CHROMOSOME ORGANIZATION AND GENIC EXPRESSION

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During the past six years, a study of the behavior of a number of newly arisen mutable loci in maize has been undertaken. This study has provided a unique opportunity to examine the mutation process at a number of different loci in the chromosomes. For some of these loci, several independent inceptions of instability have occurred during the progress of this study. The types of mutation that appear, and the types of instability expression, need not be the same at any one locus. In fact, comparisons of the behavior of these different mutable conditions at a particular locus have shown striking diversity, not only with regard to the changes in phenotypic expression that result from mutations at the locus, but also with regard to the manner in which mutability is controlled. Knowledge of the genetic constitutions, with respect to mutable loci already present in the plants in which new mutable loci have arisen, and the subsequent behavior of the newly arisen mutable loci, have provided evidence that allows an interpretation of their mode of origin and also their mode of operation. As a consequence of this study, some rather unorthodox conclusions have been drawn regarding the mechanisms responsible for mutations arising at these loci. The same mechanisms may well be responsible for the origins of many of the observed mutations in plants and animals.

Instability of various loci—whether referred to by the terms mutable loci, mutable genes, or variegation, position effect, etc.—has been known for many years, and many such cases have received considerable study. The conditions associated with the more obvious position-effect phenomena in *Drosophila* are well known. Those associated with instability of phenotypic expressions in other organisms have been less well understood. It is because of the distinctive advantages that the maize plant offers for such a study that it has been possible to obtain precise evidence concerning some of the events associated with the origin and behavior of mutable loci. The first of these advantages relates to the ease of observing the chromosomes, and thus determining the nature of some of the changes that occur in them. The presence of a triploid endosperm in the kernel provides a second advantage. This endosperm, with its outer aleurone layer that can develop pigments, and the underlying tissues that may develop starches of several types, or sugars, or carotenoid pigments, permits the detection of differences in phenotypic expression of various types. Some of these may be quantitatively measured. Thirdly, there are a number of different loci known in which heritable alterations have given rise to changes in the expression of these several endosperm components. The mutations at some of these loci affect characters of both the endosperm and the plant tissues. This applies particularly to those mutations that affect the development of the anthocyanin pigments. In the studies to be described, the presence in the short arm of chromosome 9 of four marked loci that affect endosperm characters has been of particular importance for analyzing the events occurring at mutable loci. The necessity of having such markers will become evident in the discussion. For this study, the accumulated knowledge of the behavior of newly broken ends of chromosomes in maize has been of particular importance. Its significance for interpreting the origin of mutable loci will be indicated in the sections that follow.

The Chromatid and Chromosome Types of Breakage-Fusion-Bridge Cycle

The diagrams of Figure 1 illustrate the mode of origin of newly broken ends of chromosomes at a meiotic mitosis and the subsequent behavior of these ends in successive mitotic cycles. A chromosome with a newly broken end entering a telophase nucleus in the gametophytic or endosperm tissues will give rise in the next anaphase to a chromatid bridge configuration (McClintock, 1941). The bridge is produced because fusion occurs between sister chromatids at the position of previous anaphase breakage. This sequence of anaphase breaks and sister-chromatid fusions will continue in successive mitoses. It has therefore been designated the chromatid type of breakage-fusion-bridge cycle. This cycle is illustrated in A of Figure 1. In the sporophytic tissues, however, this cycle usually does not occur. The broken end entering a telophase

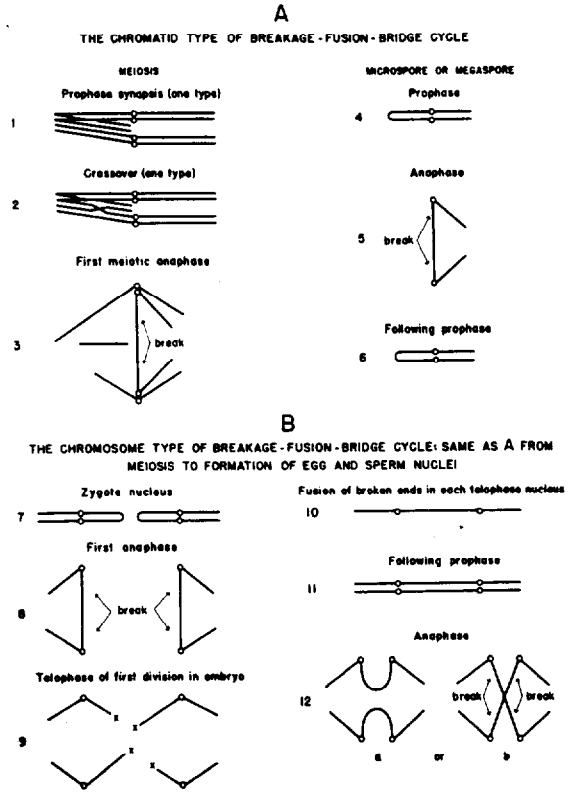


FIG. 1. Diagrams illustrating the origin of a newly broken end of a chromosome at the meiotic anaphase and its subsequent behavior. A. The chromatid type of breakage-fusion-bridge cycle. 1. One type of synaptic configuration at the first meiotic prophase between homologous arms of a pair of chromosomes, one member of which carries a duplication of this arm in the inverted order. 2. The production of a dicentric chromatid as the consequence of a crossover. It is composed of two complete chromatids of this chromosome. 3. Anaphase *I*. Bridge configuration produced by separation of centromeres of the dicentric chromatid. A break in the bridge occurs at some position between the two centromeres. 4. Fusion of sister chromatids at the position of the previous anaphase break is exhibited in prophase of the microspore or megaspore nucleus. 5. Separation of sister centromeres at anaphase in the microspore or megaspore produces a bridge configuration. This bridge is broken at some position between the two centromeres. 6. Fusion of sister chromatids occurs at the position of the preceding anaphase break. Separation of sister centromeres at the next anaphase again produces a bridge which is broken at some position between the two centromeres. This cycle continues in successive mitoses during the development of the gametophyte and the endosperm.

B. The chromosome type of breakage-fusion-bridge cycle. It may be initiated in the sporophyte if each gamete contributes a chromosome which has been broken in the anaphase of the division preceding gamete formation. The zygote nucleus will then contain two such chromosomes. In the prophase of the first division of the zygote, 7, each of these is composed of two sister chromatids fused at the position of the previous anaphase break. In the first anaphase of the zygotic nucleus heals, and its subsequent behavior resembles that of a normal, nonbroken end of a chromosome. (Note: The chromatid type of breakage-fusion-bridge cycle can continue throughout the development of the sporophytic tissues under certain conditions. These conditions are usually not present in the genetic stocks of maize.) If, however, a chromosome with a newly broken end is introduced into the zygote by each gamete nucleus, the broken ends of the two chromosomes are capable of fusion (McClintock, 1942). This establishes a dicentric chromosome. A different type of breakage-fusion-bridge cycle is thereby initiated. In the telophase nuclei, the fusions now occur between the broken ends of chromosomes rather than between the broken ends of sister chromatids, as described above. This sequence of events has been called the chromosome type of breakage-fusion-bridge cycle, and is illustrated in B of Figure 1. A study of the consequences of these cycles has revealed that they may initiate breakage events in chromosomes of the complement other than those undergoing the cycle. This complication has been of significance, for it appears that these unanticipated alterations of the chromosomes may be responsible primarily for the origin of mutable loci and of other types of heritable change.

Unexpected Chromosomal Aberrations Induced by the Breakage-Fusion-Bridge Cycles

In the course of an experiment designed to induce small internal deficiencies within the short arm of chromosome 9, a number of plants were obtained that had undergone the chromosome type of breakage-fusion-bridge cycle in their early developmental period. The short arm of each chromosome 9 was involved in this cycle. It is

division, 8, these two chromosomes give rise to bridge configurations as the centromeres of the sister chromatids pass to opposite poles. Breaks occur in each bridge at some position between the centromeres. In the telophase nuclei, two chromosomes, each with a newly broken end, are present as diagrammed in 9. The crosses mark the broken ends of each chromosome. Fusion of broken ends of *chromosomes* occurs in each telophase nucleus, 10, establishing a dicentric chromosome. In the next prophase, 11, each sister chromatid is dicentric. At the subsequent anaphase, several types of configurations may result from separation of the sister centromeres, two of which are shown in 12. Separations as shown in *b* of 12 give rise to anaphase bridge configurations. Breaks occur in each bridge at some position between the centromeres. The subsequent behavior of the broken ends, from telophase to telophase, is the same as that given in diagrams 9 to 12.

known that the cycle will often cease suddenly in certain cells and that these cells are then capable of developing sexually functional branches of the plant. In order to determine the nature of the chromosome changes produced by this cycle, the sporocytes of many of these plants were examined at the pachytene of meiosis. The expected types of altered constitution of the short arm of chromosomes 9 were found. In addition, other quite unexpected types of chromosome aberration appeared in a number of the plants. These alterations had been produced in the early developmental periods when the breakage-fusion-bridge cycles were occurring. With a few exceptions, the chromosome parts in which alterations had been initiated were the knobs and the centromeres, or the nucleolus organizer of chromosome 6. In the majority of cases, either the knob or the centromere of one of the chromosomes 9 that had been undergoing the breakage-fusion-bridge cycle was involved in the structural rearrangement. Nonrandomness was apparent with regard to the other chromosome involved in the aberration. For example, four cases were found in which the centromere of chromosome 9 had fused with the centromere of another chromosome—chromosome 2 in three of the four cases. Chromosome 8 was also very frequently involved in these structural changes.

The breakage-fusion-bridge cycle was obviously responsible for the induction of these alterations in the knobs, centromeres and the nucleolus organizer. That alterations in such elements were occurring without obvious direct participation of the knob or the centromere of the chromosome 9 undergoing the breakage-fusion-bridge cycle has also been indicated. This was made evident by the presence in one plant of an inversion involving the nucleolus organizer and the centromere region in chromosome 6, by an inversion in chromosome 5 in another plant involving the centromere and the knob regions, and by an inversion in chromosome 7 in a third plant involving the centromere region and the knob region in the long arm of this chromosome. In addition, some of the plants examined showed the presence of a ring chromosome that was not composed of segments of chromosome 9, so far as could be determined. It now must be emphasized that it was in the self-pollinated progeny of plants that had undergone the chromosome type of breakage-fusion-bridge cycle in their early developmental period that the initial burst of newly arisen mutable loci appeared. It might be suspected that this burst was a reflection of the mechanism that had produced the alterations mentioned above. If so, the origin of mutable loci would be associated with change in these particular elements of the chromosome complement. It was some time, however, before sufficient evidence had accumulated to allow deductions to be drawn regarding this presumptive relationship. A description of the origin and behavior of some of the representative types of mutable loci should be given before this topic is again considered.

Recognition of the Relation of Mutation to the Mitotic Cycle

Interest in these mutable loci, appearing unexpectedly and in large numbers in the self-pollinated progeny of plants that had undergone the chromosome type of breakage-fusion-bridge cycle in their early developmental periods, was aroused when it was realized that in each case some factor was present which controlled the time or the frequency of mutations. This factor could be altered as a consequence of some event associated with the mitotic process. This was made evident by the appearance of sectors of tissue, derived from sister cells, that exhibited obvious differences in time of mutations, mutation frequency, or both. In many cases, it was also apparent that the mutations themselves arose as a consequence of some event associated with the mitotic cycle. This basic behavior pattern was exhibited by all the various newly arisen mutable loci. It directed attention to the mitotic mechanism as the responsible agent. It was concluded, therefore, that further investigation of these mutable loci might produce some evidence leading to an appreciation of the nature of the responsible mitotic events.

During six years of study of a number of newly arisen mutable loci, some well-established facts have accumulated concerning the processes associated with the origin of mutable loci and their subsequent behavior. Observation of consistent behavior in many mutable loci, where the cytological events associated with a change in phenotype could be determined, and comparison of the behavior of these loci with others in which cytological determinations could not readily be made, have provided an assemblage of interrelated facts upon which the conclusions to be stated later are based.

The Origin of *Ds* and its Behavior

The first evidence of the type of chromosomal event that is associated with the expression of mutability came with the discovery of a locus in

the short arm of chromosome 9 at which chromosome breaks were occurring. This was observed in the self-pollinated progeny of one of the plants that had undergone the chromosome type of breakage-fusion-bridge cycle in early development. When first seen, the "mutability" was expressed by the time and frequency of the breaks that occurred at this locus in some cells during the development of a tissue. Also, some change could occur in somatic cells that affected the time and frequency; and this latter event likewise was associated with the mitotic process. The behavior pattern resembled in considerable detail the patterns exhibited by the mutable loci. In this case, however, a mechanism associated with chromosome fusion and subsequent breakage was responsible for the behavior observed. The mutations from recessive to dominant exhibited by the mutable loci would not alone have suggested a chromosome-breakage mechanism as being responsible. Because of this similarity of the patterns of behavior, it was suspected that the basic mechanism responsible for mutations at mutable loci could be one associated with some form of structural alteration at the locus showing the mutation phenomenon. This conclusion was consistent with the very first observations of the behavior of mutable loci. These observations had indicated that the events at mutable loci leading to mutations and also other events controlling their time and frequency of occurrence were associated with alterations that were in some manner produced during the course of a mitotic cycle.

Intensive study of this locus in chromosome 9 at which structural alterations occur at regulated rates and at regulated times in development has been rewarding. A "break" in the chromosome at this locus was the event first recognized. The factor responsible was therefore given the symbol *Ds*, for "Dissociation." The nature of the breakage event was later determined. It arises from dicentric and acentric chromatid formations. The acentric fragment is composed of the two sister chromatids, from the *Ds* locus to the end of the short arm. The complementary dicentric component includes the sister segments from the locus to the centromere plus the long arms of the two sister chromatids. This is the type of recognizable event found to occur most frequently at *Ds*. Other recognizable aberrations, however, may sometimes arise. One of them is the formation of an internal deficiency in the short arm of chromosome 9. Such deficiencies include the regions adjacent to *Ds*, and vary in extent from minute to quite large. Translocations between this chromosome and another chromosome of the complement may arise, with one of the points of breakage at the *Ds* locus. Duplications, or inversions, of segments within chromosome 9 may also be produced, one of the breakage points being at *Ds*.

It was realized early in this study of *Ds* that changes could occur at the locus leading to marked alterations in frequency of the detectable breakage events. The original isolate was showing high frequencies of formation of dicentric chromatids and the associated acentric fragments. Changes arose at the locus, however, as a consequence of some event occurring in a somatic cell. These changes resulted in the appearance, in subsequent cell and plant generations, of lowered frequencies of these events. Such changes in the behavior pattern of *Ds* were called "changes in state"; and the *Ds* with the altered state behaved in inheritance as an allele of the original isolate of *Ds*. A subsequent change could occur, which again was recognized by an altered frequency of detectable breakage events, and which behaved in inheritance as an allele of the initial state, of the derived state, or of other unrelated derived states. By selecting altered states of *Ds*, a series of alleles of the original *Ds* has been isolated. The changes in state of *Ds*, and those occurring at other mutable loci, are of considerable significance in understanding the nature of the events responsible for the patterns of behavior of all mutable loci. A discussion of this significance will be postponed until the behavior of some other mutable loci have been considered. The meaning of the term will then be readily apparent.

TRANSPOSITION OF *Ds*

An important aspect of this study, with regard to the origin of mutable loci and nature of their mutation process, is related to transposition of *Ds* from one location in the chromosome complement to another. The discovery of such transpositions occurred in the course of studies aimed at determining the exact location of *Ds* in chromosome 9. These tests involved linkage relationships. A sequence of six marked loci along the chromosome arm were used, and the linkage studies clearly established the location of *Ds* as shown in Figure 2. This genetically determined location fitted the position of breaks in the chromosome observed in some of the sporo-

cytes of plants having *Ds* in either one or both chromosomes 9. Such chromosome breaks are illustrated in the photographs of microsporocytes at pachytene given in Figures 4 to 8. This was the location of *Ds* when it was first discovered, and has been called the standard location.

In the course of studies of the inheritance behavior of *Ds*, an occasional kernel appeared which showed that *Ds*-type activity—that is, chromosome breakage—was occurring at a new position in the short arm of chromosome 9. Attempts were made to germinate such kernels when they were found. If a plant arose from one, a study was then commenced to determine the new location of the *Ds*-type activity. Over 20 cases of the sudden appearance of *Ds*-type activity in new locations in the short arm of chromosome 9, and several cases of its sudden appearance in other chromosomes of the complement, have been investigated. Within the short arm of chromosome 9, such activity has appeared at various positions. All the isolates studied have shown sharply defined locations of the *Ds*-type activity. In these cases, the cytological determination of breakage position and the genetic determination of location were in agreement. New positions of *Ds*-type activity have appeared between all of the marked loci shown in Figure 2. For example, in four independently arisen cases, the new position of *Ds* has been located between *I* and *Sh*. In two of these, it is to the right of *I*, at or close to the same position in each case—approximately one-fifth the crossover distance between *I* and *Sh*. In the other two it is to the left of *Sh*, with a very low percentage of crossing over between *Ds* and *Sh* in each case.

The mode of detecting new locations of *Ds*-type activity has been selective, in that those arising in the short arm of chromosome 9 are immediately revealed on many of the ears coming from test crosses. *Ds*-type activity has suddenly appeared, however, in other chromosomes of the complement. Only when appropriate genetic markers are present can it be detected readily; and in most tests, such markers have not been present.

Several questions must now be asked. How do new positions of *Ds* activity arise? And what conditions are responsible for their occurrence? The methods used in seeking answers to these questions may be described. In some cases, it could be established that the appearance of *Ds* activity at a new location was associated with its disappearance at the known former location. It has been emphasized that the mechanism underlying *Ds* events is one that can give rise to translocations, deficiencies, inversions, ring-chromosomes, etc., as well as the more frequently occurring dicentric chromatid formations with reciprocal formation of acentric fragments. It has also been stated that in each such case one breakage point is at the known location of *Ds*. The appearance of *Ds* at new locations is probably associated with such a break-inducing mechanism. This was indicated by extensive analysis of the constitutions of two independent duplications of segments of the short arm of chromosome 9 when a new location of *Ds* activity was also present in this arm. In both cases, only one of the many tested gametes of one of the parent plants carried the particular chromosome aberration with the new location of *Ds*. It was detected in two single aberrant kernels on separate ears coming from similar types of crosses made in two different years. The female parent carried two morphologically normal chromosomes 9, each with the markers *C*, *sh*, *bz*, and *wx*. No *Ds* (or *Ac*, see below) was present in these plants. The male parent (one *Ac* present) carried two morphologically normal chromosomes 9. The markers *I*, *Sh*, *Bz*, *Wx*, and *Ds* (at its standard location) were present in one chromosome 9. The homologous chromosome carried *C*, *sh*, *bz*, *wx*, but no

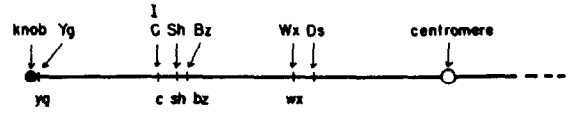
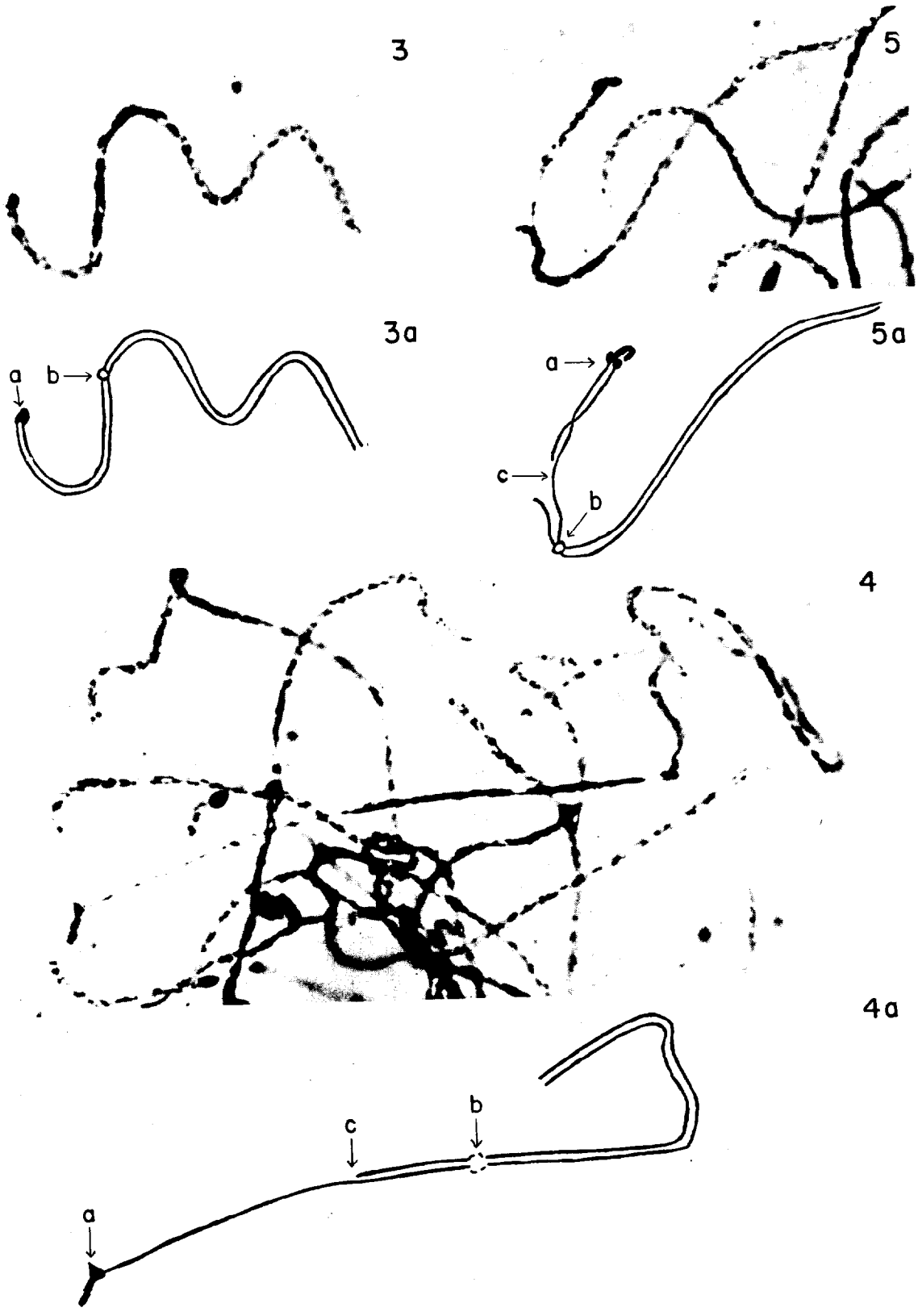


FIG. 2. Diagram showing the approximate locations of the genetic markers in the short arm of chromosome 9 that have been used in this study. In symbolization, dominance is indicated by a capital letter or capitalization of the first letter. Recessiveness is indicated by lower-case letters. The symbols refer to the following plant or endosperm characters: *Yg*, normal chlorophyll; *yg*, yellow-green chlorophyll color in early period of development of the plant. *Sh*, normal endosperm; *sh*, shrunken endosperm. *I*, *C*, and *c* form an allelic series associated with pigment development in the aleurone layer of the endosperm. *I*, inhibitor of aleurone color formation, dominant to *C*. *C*, aleurone color, dominant to *c*, colorless aleurone. The *Bz* factor is associated with development of aleurone and plant color. When homozygous, the recessive, *bz*, (bronze), gives rise to an altered anthocyanin color in the aleurone and plant tissues, from a dark red or purple to a bronze shade. When *Wx* is present, the starch in the pollen and endosperm stains blue with iodine solutions, due to the presence of amylose starch; when only the recessive *wx* (waxy) is present, no amylose starch is formed and with iodine solutions, the starch stains a reddish-brown color. The position of *Ds*, indicated in the diagram, is the standard location (see text).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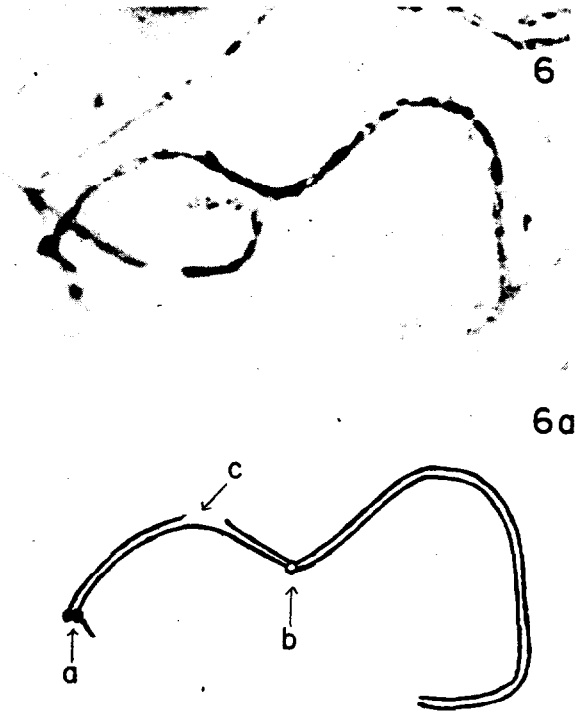
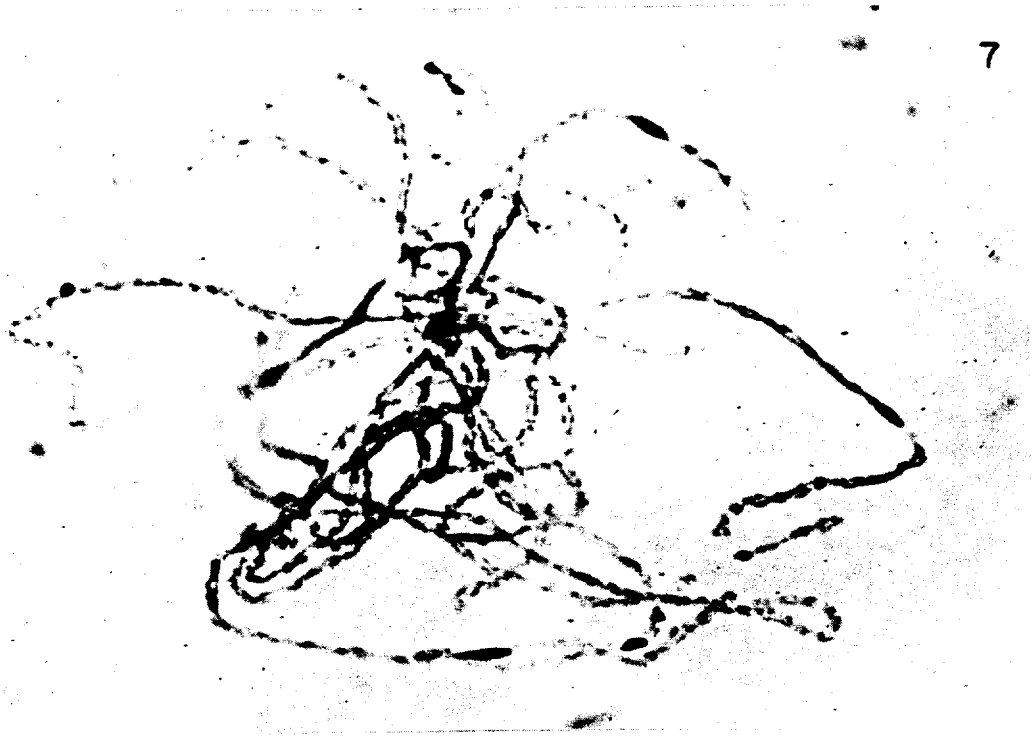


FIG. 3. Photograph of a normal bivalent chromosome 9 at pachytene of meiosis. In the accompanying diagram, 3a, the knob terminating the short arm is indicated by the arrow, *a*. The centromere is indicated by the arrow *b*. Mag. approximately 1800x. Fusion of homologous centromeres appears to occur at pachytene. Consequently, in the diagrams accompanying Figures 3 to 8, this region is indicated as single rather than double.

FIGS. 4 to 7 and accompanying diagrams, 4*a* to 7*a*. Illustrations of the position of breaks at the *Ds* locus as seen at pachytene of meiosis in plants having *Ds* at its standard location in one chromosome 9 and no *Ds* in the homologue. The two homologues are distinguishable. At the end of the short arm of the chromosome 9 having no *Ds*, a segment of deep-staining chromatin extends beyond the knob. The short arm of the chromosome 9 carrying *Ds* terminates in a knob. Magnifications approximately 1800x. In Figure 4, a break at *Ds* occurred in a premeiotic mitosis. The acentric fragment, from *Ds* to the end of the arm, was lost to the nucleus. Consequently, this segment is missing in the bivalent. The homologous segment in the chromosome 9 having no *Ds* is therefore univalent. In making the preparation, this segment was considerably stretched. In the accompanying diagram, arrow *a* points to the knob and the small deep staining segment extending beyond the knob. Arrow *b* points to the centromere region, not clearly shown in the photograph. Arrow c points to the position of the break in the chromosome 9 that carried *Ds*. Figures 5 and 6 show the appearance of the bivalent chromosome 9 when a break in the *Ds* carrying chromosome occurred at the meiotic prophase and when the free segment, from *Ds* to the end of the arm, paired with its homologous segment in the chromosome 9 having no *Ds*. In the accompanying diagrams, arrow *a* points to the knobs, arrow *b* points to the centromeres and arrow *c* to the position of the *Ds* break in one of the homologues. Figure 7 is similar to Figures 5 and 6 except that the free fragment, from the position of *Ds* to the end of the arm, did not pair with its homologous segment in the chromosome 9 having no *Ds*. In the accompanying diagram, arrow a^1 points to the knob and the deep-staining chromatin extending beyond the knob in the chromosome having no *Ds*. Arrow a^2 points to the knob of the unpaired acentric fragment. Arrow *b* points to the position of the centromeres, not observable in the photograph. Arrow c^1 points to the broken end of the centric segment, and arrow c^2 points to the broken end of the acentric segment. (For Fig. 7, see next page.)



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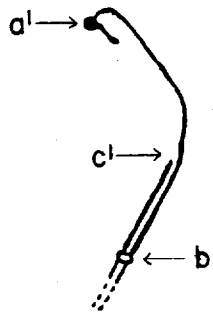

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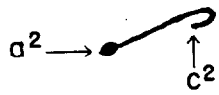


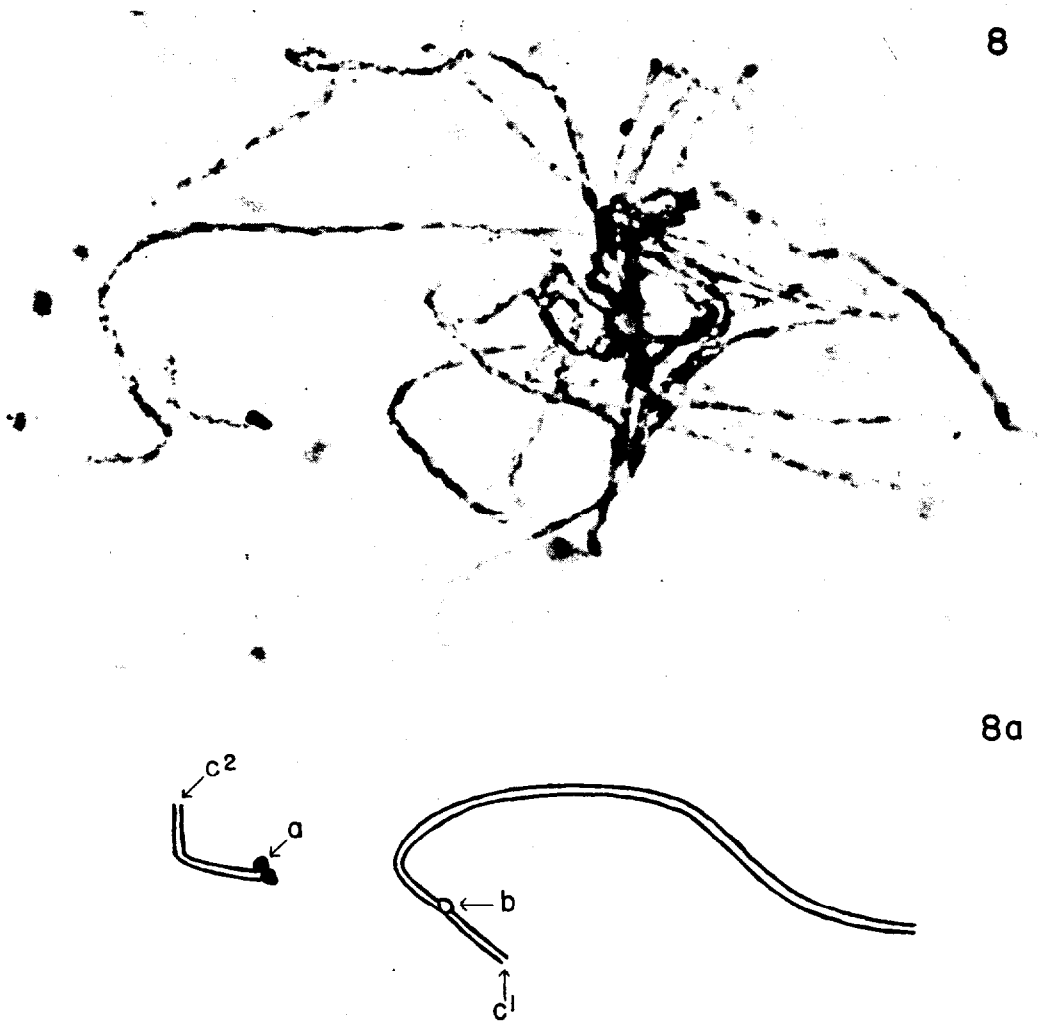


FIG. 8. The chromosome 9 bivalent at pachytene in a plant having *Ds* at its standard location in each chromosome 9. A *Ds* break occurred at the meiotic prophase in each chromosome. Consequently, the bivalent is composed of two free segments: one short acentric segment, from *Ds* to the end of the arm, and a long centric segment, from the position of *Ds* to the end of the long arm. In the accompanying diagram, arrow *a* points to the large knob terminating the short arm of each member of the acentric bivalent segment, and arrow *b* points to the centromeres of the centric bivalent segment. Arrow c^1 points to the broken ends of the centric bivalent segment and arrow c^2 points to the broken ends of the acentric bivalent segment.

Ds. The constitutions of the aberrant chromosomes 9, which were present in an individual pollen grain in each case, are shown in Figure 9. Each has a duplication of a segment of the short arm of chromosome 9. A study of these constitutions reveals that, in each case, the new position of *Ds* activity coincided with the position of one of the breaks that produced the duplication. Also, the position of the second break coincided with the previously known location of *Ds* in the morphologically normal *Ds*-carrying chromosome 9 of the male parent plant. It seems clear from the analysis of both these cases that the breaks were *Ds* initiated, and also that both breaks involved sister chromatids at the same location. Two other cases of newly arisen duplications associated with new positions of *Ds* activity have received study. These have proved to be similar in their modes of origin to the two cases diagrammed. Although these cases suggest that the new positions of *Ds* activity may arise from contacts with the *Ds* that is present in the chromosome complement, they do not constitute evidence that *Ds* is composed of a material substance and that the new positions arise from insertion of this material into new locations.

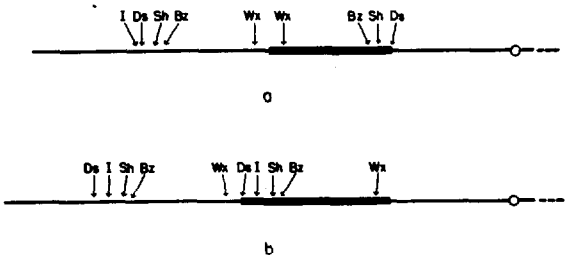


FIG. 9. Diagrams illustrating the constitution of two chromosomes 9, each of which carries a duplicated segment (heavy line). A transposition of *Ds* accompanied the formation of the duplication in each case. For details, see text.

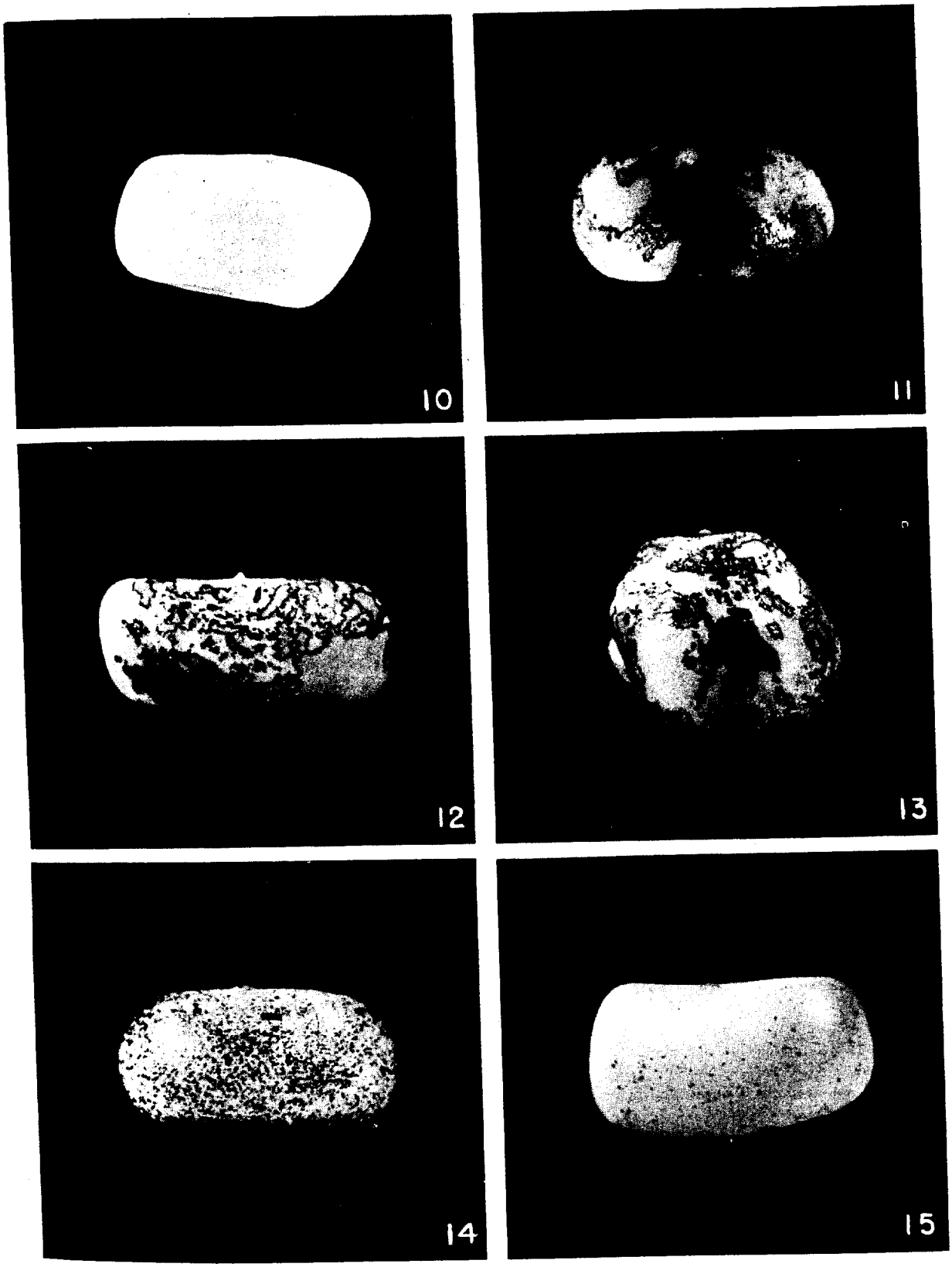
Ac Behavior and Inheritance: General Considerations

Before continuing discussion of the sudden appearance of *Ds* at new locations, it is necessary to consider another heritable factor called Activator (*Ac*). *Ac* is a dominant factor that must be present if any breakage events are to occur at *Ds*. If *Ac* is absent from the nucleus, no detectable events whatsoever occur at *Ds*, wherever it may be located; and no new positions of *Ds* activity appear. Because of this, and because new positions of *Ds* arise only in a few cells, during the period of development of a tissue when breakage events are occurring at *Ds*, it may be concluded that these new positions are one of the consequences of the mechanism that produces chromosome breakage events at *Ds*.

The location of *Ds* in chromosome 9 was first suggested by the altered phenotype of sectors of tissue derived from cells in which a break had occurred at *Ds*. Such sectors will appear if the factorial constitutions of the homologues differ. The chromosome arm carrying *Ds* must also carry dominant markers, and the homologue must carry the recessive alleles. If one chromosome 9, delivered by the male parent, carries *I*, *Sh*, *Bz*, *Wx*, and *Ds* at its standard location, and if the homologue delivered by the female parent carries the recessive alleles *C*, *sh*, *bz*, and *wx*, and has no *Ds*, the events at *Ds* that form a dicentric chromatid and an acentric fragment will lead to elimination of the acentric fragment during a mitotic division. This fragment will form a pycnotic body in the cytoplasm, which subsequently disappears. All the dominant markers carried in this acentric fragment will be removed from the nuclei after such an event has occurred. The sector of tissue derived from these cells will exhibit the collective phenotype of the recessive alleles carried by the homologous chromosome 9 having no *Ds*.

If pollen of plants having one *Ac* factor and carrying *I*, *Sh*, *Bz*, *Wx*, and *Ds* (standard location) is placed upon silks of plants carrying *C*, *sh*, *bz*, and *wx* in their chromosomes 9, but having no *Ds* or *Ac*, two types of kernels will appear, those that received an *Ac* factor and those without *Ac*. The latter kernels will be colorless and nonshrunken, and will show the *Wx* phenotype. No variegation for the characters exhibited by the recessive alleles will appear, as shown in Figure 10. If *Ac* is present in the endosperm, however, the described *Ds* events will occur in some cells during the development of the kernel. This will result in elimination of the segment in the short arm of chromosome 9 carrying the dominant factors. All the cells arising from one in which such an event has occurred will exhibit the *C*, *sh*, *bz*, and *wx* phenotypes. Consequently, these kernels will be variegated. The photographs of kernels in Figures 11 to 13 will illustrate the nature of this variegation.

FIGS. 10 to 15. Photographs of kernels illustrating the effects produced by breaks at *Ds*, and the relation of the presence or absence of *Ac* and the doses of *Ac* on the time of occurrence of *Ds* breaks. The kernels arose from the cross of a plant (♀) carrying *C* and *bz* and having no *Ds* in each chromosome 9, by plants (♂) having *I*, *Bz*, and *Ds* at its standard location in chromosome 9. In Figure 10, no *Ac* is present; the kernel is completely colorless due to the inhibition of aleurone color when *I* is present. In Figures 11 to 13, one *Ac* is present. Breaks at *Ds* occur early in development with consequent loss of *I* and *Bz* to the nuclei. The cells without *I* and *Bz* give rise to sectors showing the *C bz* phenotype. It should be noted that *Bz* substance diffuses through several cell layers, from the *I Bz* areas into the *C bz* areas, producing a *C Bz* phenotypic expression in these genotypically *C bz* cells. Thus, all large areas of the *C bz* genotype are rimmed with the dark aleurone color of the *C Bz* phenotype. Small *C bz* areas may be mostly *C Bz* in phenotype because of this diffusion, and very small *C bz* areas are totally *C Bz* in phenotype. These relationships are clearly expressed in the photographs. In Figures 11 to 13, both large and small *C bz* sectors are present as well as areas in which no *C bz* sectors appear. This irregularity arises from alterations that occur to *Ac* during the development of the kernels. These give rise to sectors with no *Ac* or with altered doses of *Ac* (see text). Figure 14 shows the pattern that may be produced by *Ds* breaks when two *Ac* factors are introduced into the primary endosperm nucleus. There are many small sectors of the *C bz* genotype, all produced by relatively late occurring *Ds* breaks. This results in a heavily speckled variegation pattern. Figure 15 shows the pattern that may appear when three *Ac* factors are present. Only relatively few *C bz* specks, produced by late occurring *Ds* breaks, are present in this kernel.


10
11
12
13
14
15

In the early studies, it soon became apparent that the time of occurrence of *Ds* breakage events in the development of a tissue depends upon the dose of *Ac* present. The higher the dose of *Ac*, the later in development will such events at *Ds* occur. Because the endosperm is triploid—the female parent contributing two haploid nuclei derived from the female gametophyte, and the male contributing one haploid nucleus—single to triple doses of *Ac* may readily be obtained after appropriate crosses. The time of response of *Ds* to double and triple doses of *Ac* is shown in Figures 14 and 15.

Initial studies of the inheritance behavior of *Ac* showed that it follows the mendelian laws known to apply to single genetic units. An illustration of this inheritance behavior is given in Figure 16. The ear in this figure was derived from a cross in which the female parent carried the recessive factor *c* in each chromosome 9, but had no *Ds* or *Ac* factors, and the male parent carried in each chromosome 9 the factors *C* and *Ds* (standard location). It also carried a single *Ac* factor. The expected 1-to-1 ratio of colored, nonvariegated kernels (no *Ac* present) to kernels showing sectors of the *c* phenotype (*Ac* present) was apparent on this ear. Efforts to determine the location of *Ac* in the chromosome complement were commenced, but were soon abandoned when it was realized that *Ac* need not remain at any one location in the complement. It can appear at new locations and in different chromosomes. Because of this highly unexpected behavior of a genetic factor, extensive studies were made to determine the mode of inheritance of *Ac* and to learn how these new positions arise. Much has been learned from them about *Ac* behavior and inheritance. The modes of investigating alterations of *Ac* will be considered later. Some of the facts concerning its behavior, however, may be stated here in summary form, by describing the results obtained from several types of experiments.

If plants having one *Ac* factor are self-pollinated, the expected mendelian ratios may appear in the F_2 populations. These are: one with two *Ac*, to two with one *Ac*, to one with no *Ac*. The ratios obtained in one such test were 61 : 145 : 68, which is close to the statistical expectancy. When, however, these F_2 plants having two *Ac* factors are crossed by plants having no *Ac*, the expectation would be that all the progeny would have one *Ac* factor. Usually, most of the plants do have one *Ac* factor; but sometimes there are plants displaying other conditions with respect

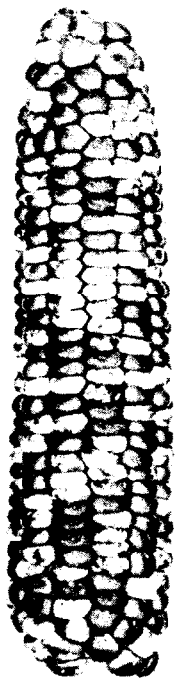


FIG. 16. Photograph of an ear produced when pollen from plants having *C* and *Ds* (standard location) in each chromosome 9 and carrying one *Ac* factor is placed on silks of plants carrying *c* in each chromosome 9 and having no *Ds* or *Ac* factors. Approximately half of the male gametes have no *Ac* factor. The kernels arising from the functioning of such gametes are fully colored; no variegation appears. The other half of the male gametes carry *Ac*. The kernels arising from the functioning of these gametes are variegated. They show a number of sectors with the *c* phenotype. Note the approximate 1 to 1 ratio of fully colored kernels to variegated kernels.

to *Ac*. The following unexpected types have appeared: plants having no *Ac* factor; others having two nonlinked *Ac* factors; others having two *Ac* factors that appear to be linked; still others having an *Ac* factor that acts as a single unit in inheritance but gives the same dosage action as would two doses of the *Ac* factor in the parent plant that contributed *Ac*. The dosage action of *Ac* may be altered in other ways, moreover, so that a single *Ac* factor, as determined genetically, may exert an action either less than that of the *Ac* factor contributed by the *Ac*-carrying parent plant or falling between one and two doses of this factor.

In the early studies of *Ac* inheritance, the *Ac* factor was found not to be linked with the genetic markers in the short arm of chromosome 9. In one series of tests of *Ac* inheritance, where a number of *Ac*-carrying plants were derived from a cross between a plant having no *Ac* and a plant

having one *Ac*, a single aberrant plant was present in the F_1. Tests of this plant showed that it possessed a single *Ac* factor, which was obviously linked to the markers in the chromosome 9 delivered by the *Ac*-carrying parent plant. In the sister plants, and in the parent plant that contributed *Ac*, no such linkage was evident. Studies were then conducted to examine the linkage relationships of this *Ac* with the marked loci. The very same types of tests for linkage of *Ac* with the genetic markers in chromosome 9 were also conducted with the sister plants. A summary of one set of these comparative tests of *Ac* inheritance is presented in Table 1. In part I of this table, no linkage of *Ac* with *I* or *Wx* is evident; in part II, however, linkage is obvious. The data place *Ac* to the right of *Wx*. Approximately 20 per cent crossing-over appears to have occurred between *Wx* and *Ac*. Actually, this figure is only approximate, for a few kernels in the *Ac-wx* class do not carry *Ac* in chromosome 9. They do not belong in the crossover class. The *Ac* in these kernels had been transposed from chromosome 9 to another chromosome. Also, a few kernels in the *Wx*-no *Ac* class have not lost *Ac* because of crossing over but rather because it was removed from its location in chromosome 9.

The described case of sudden appearance of *Ac* in chromosome 9 is not the only one that has been found and similarly studied. Seven independent cases have so far been identified. In two of these, *Ac* appeared in the short arm of this chromosome: in the first case, a short distance to the left of *Wx*; and in the second case, a short distance to the left of *I*. Studies were then undertaken to investigate not only the linkage behavior of *Ac* but also the types of events that alter *Ac* in these two sharply delimited locations. It was determined that in the majority of the *Ac*-carrying gametes produced by plants having an *Ac* at either of these two stated positions, no change in location or action of *Ac* occurred. Exchanges of *Ac* from one homologue to another took place as a consequence of crossing over, with consistent frequencies in each case. In a few of these gametes, however, the above-described types of aberrant *Ac* conditions were present, as follows: (1) *Ac* was no longer present in chromosome 9, but was carried instead by another chromosome. (2) Two *Ac* factors were present, one at the given location in chromosome 9, and one carried by another chromosome of the complement. (3) The *Ac* factor was unchanged in its location but showed an altered action; in a single dose it could be equivalent to the double dose before the alteration occurred, or could show an increased but not doubled dosage action, or a decreased dosage action.

The behavior of *Ac* was also studied in plants in which the *Ac* factor was present at an allelic position in each chromosome 9. Again, it could be determined that in a few gametes produced by such plants the above-described aberrant conditions with respect to *Ac* position and action were present. In addition, a few gametes were formed that had no *Ac* factor at all.

From what has been said about both *Ds* and *Ac*, it is apparent that with respect to inheritance

TABLE 1

Comparisons of *Ac* inheritance: (1) when *Ac* was not linked to markers in chromosome 9, and (II) when *Ac* was linked to markers in chromosome 9, in crosses of

(I) $\sigma \frac{I\ Sh\ Bz\ Wx\ Ds}{C\ Sh\ Bz\ wx\ Ds}$ 1 *Ac*, nonlinked

♀ *C sh bz wx/C sh bz wx*, no *Ds*, no *Ac* by (I) and

(II) $\sigma \frac{I\ Sh\ Bz\ Wx\ Ds\ Ac}{C\ Sh\ Bz\ wx\ Ds\ ac}$

Chromosome-9 Constitution of ♂ Gamete	I		II	
	Variegated* *Ac* present	Nonvariegated* No *Ac*	Variegated* *Ac* present	Nonvariegated* No *Ac*
I Wx	268	255	928	246
C wx	248	242	164	893
I wx	88	91	62	387
C Wx	84	83	344	100

*Presence of *Ac* detected by sectors of the *C sh bz wx* phenotype in kernels on ears obtained from cross I or cross II. In the absence of *Ac*, no *Ds* events occur; the kernels are therefore nonvariegated.

behavior they are much alike. The same questions concerning mode of appearance in new locations in the chromosome complement apply to *Ac* as to *Ds*. With this relationship in mind, we may now return to further considerations of *Ds*. It should be emphasized again, however, that the described events occurring at *Ds*, wherever it may be located in the chromosome complement, depend upon the presence of an *Ac* factor in the nucleus, regardless of where this latter factor is located in the chromosome complement. New positions of *Ds* activity arise only when *Ac* is also present in the nucleus, and, again, regardless of where *Ac* may be located. In addition, any one altered state of *Ac*—for example, an altered dosage action—affects *Ds* wherever it may be located, and in exactly the same manner for every *Ds*, regardless of its state. In other words, it is the state and the dose of *Ac* that control just when and where *Ds* events will occur, and it is the state of a particular *Ds* that controls the relative frequency of any one type of event that occurs at *Ds*.

The Origin and Behavior of c^{m-1}

In the discussion of the appearance of *Ds* at new locations, the question was raised whether or not this involves the transposition of a material substance from one location to another. The question applies equally to *Ac*. If no material substance is transposed, a serious problem is presented regarding the basic action of any known genetic unit or factor that has been assigned to a particular locus in a particular chromosome. *Ac* clearly produces an obvious, measurable, phenotypic response, wherever it may be located. It shows dosage action, mendelian inheritance, and linkage behavior of the expected type, in any location—with the exception, already mentioned, of a few transpositions, changes in state, and losses of *Ac*. It might be considered that *Ds* and *Ac* represent forms of altered chromosome organization producing somewhat similar effects in each case, much like the Minutes in *Drosophila*. The evidence now to be presented, however, makes that assumption unlikely. This evidence considers the origin and the behavior of many different mutable loci. To begin this part of the discussion, we may consider the origin of mutable c^{m-1}, the first-detected mutable *c* locus that arose in a chromosome carrying a normal-behaving *C* locus (*C*, aleurone color; *c*, recessive allele, colorless aleurone).

The presence of an alteration at the known locus of *C*, which produced c^{m-1}, was detected probably within a few nuclear generations after it occurred. It was present only in one kernel among approximately 4,000 examined that had come from a cross of a single plant, used as a male, to 12 genetically similar female plants. All the other kernels on these ears gave the expected types of phenotypic expression. The male parent carried in both chromosomes 9 the genetic markers *Yg*, *C*, *Sh*, *wx*, and *Ds* (standard location). It also had one *Ac* factor, not linked to these markers. The female parents carried the stable recessive *yg*, *c*, *sh*, and *wx*. No *Ds* was present in their chromosomes 9, and also no *Ac* factor was present in these plants. The types of kernels to be expected from such a cross, and their relative frequencies, are the same as those shown in Figure 16. Approximately half the kernels should show the *C Sh wx* phenotype, with no variegation for the recessive characters since no *Ac* factor is present. The other half should carry *Ac* and thus be variegated. Sectors showing the *c sh* phenotype should be present. In these crosses, the expected classes of kernels appeared with the exception of one kernel. Instead of showing colorless areas in a colored background (resulting from losses of *C* following breakage events at *Ds*), this exceptional kernel showed a colorless background in which colored areas were present. The plant derived from this kernel was tested in various ways in order to determine the reason for this unexpected type of variegation. The early tests indicated, and subsequent tests proved, that mutations were occurring from the recessive *c*, to the dominant *C*, and that the mutable condition had arisen in one of the chromosomes 9 contributed by the *Ac*-carrying male parent plant.

Ds-type activity was also present in the chromosome carrying the new mutable *c* locus. The location of this activity was no longer to the right of *wx*, as would be expected since this was its location in both chromosomes 9 of the male parent plant. The new location was inseparable from that of the mutable *c*. All the recognizable breakage events associated with *Ds*-type activity now happened at this new location. In addition, mutations to *C* occurred. It was soon discovered that the mutations to *C* would appear only if *Ac* were also present in the nucleus, and that the time of occurrence of these mutations was controlled by the state and dose of *Ac* in precisely the same way that the state and dose of *Ac* controls *Ds* breakage events wherever *Ds* may be located. If *Ac* were absent, neither mutations to *C* nor *Ds*-type breakage events would occur. Thus when *Ac* is absent, the behavior of c^{m-1}

is equivalent to the previously known recessive *c*. If, however, *Ac* is again introduced into the nucleus by appropriate crosses, the potential mutability of this recessive is realized, for then mutations to *C* occur. The previously known recessive *c*, used for many years in genetic studies, is unaffected by the presence of *Ac*; and it remains stable, nonmutable, when present in nuclei in which c^{m-1} is also present and undergoing mutations.

In considering the mode of origin of c^{m-1} and its behavior, the following points may be reviewed: (1) the appearance of a new recessive that is mutable; (2) its derivation from a normal dominant *C*, which is nonmutable; (3) its appearance in a single gamete of a plant carrying *Ds* and *Ac*; (4) *Ac* control of the new mutable condition in exactly the same manner as *Ds* is *Ac*-controlled; (5) *Ds*-type chromosome breakage events also occurring at this mutable locus; and (6) disappearance of *Ds* from its former location in the same chromosome that carries the new mutable locus. This series of coincidences is striking enough in itself to command consideration of the possibility that this mutable recessive originated by transposition of *Ds* to the locus of the normal *C* factor. It is immediately apparent that, if this is true, the transposition of *Ds* from its former location to the new location created a condition that affects the formation of pigment in the aleurone layer; for no pigment is formed until some event occurs at this locus, and only when *Ac* is present. Previous tests have shown that the same *c* phenotypic expression can also arise if the tissues of the endosperm are homozygous deficient for the segment of chromatin carrying *C* (McClintock, unpublished). This might suggest that the presence of *Ds* has inhibited the normal action of the chromatin materials at the *C* locus. A final and most significant argument for the origin of c^{m-1} by a transposition of *Ds* to the *C* locus is derived from the fact that a mutation to *C* is associated with the loss of any further recognizable *Ds* events at the immediate location of *C*. It is apparent, therefore, that the mutation-producing event is associated with one involving *Ds* at this locus. All the evidence is consistent with the assumption that c^{m-1} arose by transposition of *Ds* to the locus of *C*, thereby inhibiting its action, and that removal of *Ds* is associated with removal of this inhibitory effect. The restored activity at the *C* locus is permanent, and its subsequent behavior resembles that present before *Ds* activity appeared at the locus. *Ac* no longer has any effect on its action and behavior, just as it had no effect before *Ds* appeared at the *C* locus to give rise to c^{m-1}.

Additional *Ds*-Initiated Mutable Loci

Simple coincidence rather than a relationship with *Ds* might still be claimed for the origin and behavior of c^{m-1} if it were the only case of such origin and behavior. Two other cases, similar to c^{m-1} and involving another marked locus, have appeared independently in the *Ds*- and *Ac*-carrying plants. Both involve the locus in chromosome 9 associated with the bronze phenotype (see Fig. 2). These two independent cases have been designated bz^{m-1} and bz^{m-2}. The description of the types of events occurring at c^{m-1} may be applied also to bz^{m-1} and bz^{m-2}. *Ds*-type breakage events occur at the mutable locus, as well as mutations from *bz* to an apparently full *Bz* expression. Both the *Ds*-type breakage events and the mutations to *Bz* are *Ac*-controlled; for if *Ac* is absent, neither will occur. In these cases also, the time when mutations to *Bz* or chromosome breaks occur is under the control of the state and the dose of *Ac*. Again, as with *C*, previous investigations had shown that a homozygous deficiency of the segment of chromosome including the *Bz* locus will reproduce the known recessive phenotype, *bz* (McClintock, unpublished).

That the presence of *Ds* close to a marked locus in a chromosome may result in frequent changes in the phenotypic expression of the marker has been indicated. Two independent cases of transposition of *Ds* from its standard location to a position near and to the left of *Sh* have been studied (see Fig. 2). In these two similar cases, less than one-half of one per cent crossing over occurs between *Ds* and *Sh*. In both cases, however, many gametes are produced that carry a "spontaneous" mutation from *Sh* to *sh*. These mutations occur only in those chromosomes carrying *Ds* immediately to the left of *Sh*, and only in plants that also have *Ac*. If *Ac* is absent, no such mutations appear. *Ac*-controlled events, therefore, in each of these two cases where *Ds* is near and to the left of *Sh*, are responsible for this high frequency of mutation to *sh*. When such a mutation occurs, *Ds* is not always lost to the chromatid; it is sometimes still present between *C* and *Bz*. Some of these "mutations" may prove to be newly arisen mutable *sh* loci, but the tests for this mutability have not been concluded.

Knowledge gained from the cases reviewed has led to the conclusion that the appearance of *Ds* at or close to the locus of a known genetic

factor can give rise to frequent changes in the action of the factor. The initial change is to an action resembling that of the known recessive allele. In the cases of c^{m-1}, bz^{m-1}, and bz^{m-2}, a subsequent alteration produces a return to apparently full dominant expression of the factor. This common type of mutational expression in these cases is of considerable significance. Its importance will become evident in the discussion of the behavior of other newly arisen mutable loci.

Origin and Behavior of One Class of Autonomous Mutable Loci

The behavior of some other types of mutable loci may now be considered for the additional and important knowledge they have contributed to an understanding of the basic processes involved. One of them is called mutable luteus. The luteus character is distinguished by a yellowish chlorophyll expression. This mutable luteus first appeared in the progeny derived from self-pollination of one of the original plants that had undergone the breakage-fusion-bridge cycle in early development. It resulted from some alteration at a normal locus, but the position of the locus in the chromosome complement was not known. This mutable locus is characterized, first, by its autonomous behavior. It required no recognizable, separate activator factor in order to undergo the mutation phenomenon. The mutations are registered in sectors of a plant as changes in the amount of chlorophyll that is produced. Alleles arise from germinal mutations. They are characterized by various quantitative grades of chlorophyll expression. These alleles, in turn, need not be stable; some of them may mutate to give higher or lower levels of chlorophyll expression. Even an allele apparently producing the full dominant expression may be unstable for it may mutate to or towards the lowest expression, which is luteus.

In studying one aspect of the behavior of this mutable luteus locus, a number of sister plants in one culture were all self-pollinated. On a resulting ear of one, and only one, of these plants, the presence of a new mutable locus was revealed. The mutability, registered in some of the kernels on this ear, involved a factor associated with the formation of pigment in the aleurone layer. Colorless kernels were present in which mutations to color occurred. None of the ears produced by the sister plants showed the presence of such a mutable factor; all kernels on these ears had the full aleurone color. Further study of the plants derived from the variegated kernels on the aberrant ear showed that a mutable condition had arisen at a previously known locus, the A_2 locus in chromosome 5 (A_2, aleurone and plant color; a_2, recessive allele colorless aleurone, altered plant color). Before the appearance of the mutable condition in this one plant, the A_2 locus had given the normal dominant expression in both parent plants. It had shown no indication whatsoever of any instability. One parent had contributed mutable luteus to this culture. The mutable luteus locus, however, was not linked with the A_2 locus. It should be emphasized that this newly arisen mutable a_2 behaved in many respects like mutable luteus. It was autonomous; and quantitative alleles were produced, some of which, in turn, were mutable.

Another mutable locus arose in a culture derived from one having mutable luteus. It first appeared as a single aberrant kernel on one ear. This ear was produced from a cross in which a plant carrying mutable luteus was used as a female parent. The male parent was homozygous for the stable recessive a_1. (A_1, aleurone and plant color, located in chromosome 3; a_1, recessive allele, colorless aleurone and altered anthocyanin pigments in the plant.) This single aberrant kernel exhibited variegation. Sectors of colored aleurone appeared in an otherwise colorless kernel. Tests were initiated with the plant derived from this kernel, and continued with the subsequent progeny. From these tests it was learned that the aberrant kernel carried a newly arisen mutable a_1 locus, designated a_1^{m-1}, whose general behavior resembled that shown by mutable luteus. It was autonomous; it produced a series of alleles showing various grades of quantitative expression of anthocyanin, in both the aleurone and the plant; and of these alleles, in turn, some were unstable, mutating to give higher or lower levels of quantitative expression of the anthocyanin pigments in both aleurone and plant. Other important aspects of the mutation phenomenon at this locus will be considered later.

In the discussion of the *Ac*-controlled mutable loci, c^{m-1}, bz^{m-1} and bz^{m-2} that arose in *Ds-Ac* carrying plants, it was emphasized that the types of mutational response were similar. Here also, the mutational expressions of the two mutable loci that have arisen in the mutable luteus stocks are much alike, and they resemble that shown by mutable luteus itself. One further example of related mutable loci will be given. It also shows the similarity of behavior of the newly

arisen mutable locus to the one already present in the plant. The direction this discussion is taking may now be apparent. It is towards the conclusion that the type of mutation occurring at a locus is a function of the type of chromatin material that is present at the locus or is transposed to it, and does not involve changes in the components of the genes themselves. Rather, it is this chromatin that functions to control how the genic material may operate in the nuclear system. With this in mind, a third example of related origins and behaviors of mutable loci may now be considered.

ORIGIN AND BEHAVIOR OF c^{m-2} AND wx^{m-1}: TWO RELATED MUTABLE LOCI

The progeny derived from self-pollination of another one of the original plants that had undergone the breakage-fusion-bridge cycle in early development was grown, and a number of these plants again self-pollinated. On a resulting ear of one of these plants, a new mutable locus was recognized. The factor involved was again associated with the production of pigment in the aleurone layer. Some of the kernels on this ear showed colored areas in a colorless background. Beginning with the plants derived from these kernels, a study was made of the condition responsible for the variegation. This proved to be due to another new mutable locus, and involved the previously discussed *C* locus in chromosome 9. This locus in the parent plant and in the sister plants of the culture, gave the normal dominant *C* expression. The new mutable condition was designated c^{m-2}, because it was the second case that appeared in this study. The types of mutation that arise from events at c^{m-2} are strikingly different from those shown by c^{m-1}. A series of alleles, as expressed by quantitative grades of pigment formation associated with the production of at least two different precursor-type diffusible substances, is produced by mutations at c^{m-2}. The intermediate alleles are not always stable, for some of them, in turn, can mutate to alleles showing higher or lower grades of color expression.

In the course of the study of c^{m-2}, a number of crosses were made, using pollen of plants homozygous for c^{m-2} and carrying a normal dominant *Wx* factor in each chromosome 9, on silks of plants carrying a stable recessive *c* and a stable recessive *wx* in both chromosomes 9 (see Fig. 2). A single aberrant kernel appeared on one of the ears resulting from this type of cross. It showed mutations to *C* and of the c^{m-2} type, and, in addition, mutations from the *wx* to and towards the *Wx* phenotype. A plant was obtained from the kernel and a study commenced to determine the nature of this instability expression. It proved to be a new mutable *wx*, and was designated wx^{m-1}. The tests showed that it had arisen in the male parent plant, which carried c^{m-2} and normal dominant *Wx* in each chromosome 9. It was present, however, in only one of the many tested male gametes of this plant. The pattern of mutational behavior of wx^{m-1} strikingly resembled that shown by c^{m-2}. A series of quantitative alleles was produced by mutations of wx^{m-1} as registered by the amount of amylose starch produced. These alleles, in turn, could mutate to give greater or lesser amounts of amylose starch. Another endosperm character also was affected by some of the mutations of wx^{m-1}. This was expressed by an altered growth of the endosperm tissue, and accompanied some but not all of the mutations to the intermediate alleles, appearing particularly often in association with a mutation to one of the lower alleles. This accompanying mutation behaved as a dominant or a semidominant.

It is of particular significance, in comparison of the behavior of the series of mutable loci c^{m-1}, bz^{m-1}, and bz^{m-2} with that of the series c^{m-2} and wx^{m-1}, that the members of both series are controlled by the very same *Ac* factor—wherever it may be located—and in precisely the same manner, with respect to time and place of occurrence of mutations. When *Ac* is absent, no mutations occur in either series. In the latter series, mutations of the intermediate alleles also occur, but only when *Ac* is present. Because of this, it has been possible to isolate a series of quantitative alleles of *C*, and also a series of quantitative alleles of *Wx*, that are stable. Stability is maintained when *Ac* is removed. The percentages of amylose starch in the endosperm, produced by a number of the alleles arising from mutations at wx^{m-1} and freed of *Ac*, have been determined in terms of single, double, and triple doses of the particular allele. (Note: The writer is grateful to Dr. G. F. Sprague and to Dr. B. Brimhall, of Iowa State College, and also to Dr. C. O. Beckmann, of Columbia University, and Dr. R. Sager, of the Rockefeller Institute for Medical Research, for their chemical analyses of the amylose content produced by some of these mutants.) The preliminary tests suggest that alleles, falling into an almost continuous series with respect to the quantity of amylose starch they produce, may be obtained. It should

be mentioned here that, as with *C* and *Bz*, previous investigation had shown that a tissue homozygous-deficient for the *Wx* locus will give the known recessive *wx* expression (McClintock, unpublished).

In order to compare the action of *Ac* on the members of these two series of *Ac* controlled mutable loci, crosses were made combining several of them in a single plant so that they might be present together in the nuclei of a tissue. By this means, it was possible to determine that the mutations at these various mutable loci arise as a function of the state and dose of *Ac*, irrespective of which mutable locus is involved or how many such loci from the same series or from the two different series are present in the nuclei of an individual plant.

In further comparison of the behavior of the different *Ac*-controlled mutable loci, one very significant correlation may now be given. It is known that all these loci show one other common characteristic. At all of them, some chromosome-break-inducing events occur, but only when *Ac* is also present in the nucleus and only at those times in the development of a tissue where mutations leading to changes in expression of the respective phenotypic character are also occurring. Again, it has been determined that such breaks may occur at the locus of *Ac* itself. The conclusion that the mutation-producing events in these two series of related mutable loci, and also at *Ac* itself, are associated with such a chromosome-break-inducing mechanism is difficult to avoid. This relationship will be explored after consideration has been given to a comparison of the types of mutability that may arise at any one known locus in a chromosome.

The descriptions of mutable loci given so far in this discussion have shown that the type of mutability and the mode of its control are not alike for all. Nevertheless, there appear to be classes of mutable loci, the members of which show similar types of changes in phenotypic expression—that is, of mutations—and similar types of control of these mutations. It is now necessary to indicate the extent to which various types of mutability expression may arise at any one particular locus. For this purpose the *Wx*, the *C*, and the A_1 loci will be chosen as examples.

Comparisons of Types of Mutability Arising at any One Locus

a. The Wx locus

Six independent mutable conditions are known for the *Wx* locus. Five of them have arisen during the present study. One, wx^{m-1}, has been considered above. It is *Ac*-controlled, and produces quantitative alleles that may be unstable when *Ac* is present but are stable when *Ac* is absent from the nucleus. Mutations at this locus also give rise to an endosperm-growth-altering factor that is dominant in expression. The second mutable *wx*, wx^{m-2}, arose from a previously stable recessive *wx* carried in genetic stocks for many years. This mutable condition first appeared in a chromosome 9 in which a complex chromosomal rearrangement was present. Its mutations are expressed by different quantitative grades of the *Wx* phenotype. In the endosperm tissues, the sector produced by a cell in which a mutation has occurred is always markedly distorted in growth-type. The third mutable condition at this locus, wx^{m-3}, originated in a plant carrying a normal dominant *Wx* and also several mutable loci. It is autonomous, in that no separate activator factor is required for mutations to be expressed. It almost always mutates to give the full *Wx* phenotypic expression. The derived mutant giving the dominant expression is also mutable, for it produces mutants giving the full recessive expression, that is, *wx*. This recessive, in turn, may mutate again to give the full dominant expression. No altered growth conditions in the endosperm tissue accompany any of these mutations. The fourth case was recognized by a sudden change in the behavior of a previously normal *Wx* locus. It shows mutations producing various grades of quantitative expression between the full dominant and the full recessive. It is autonomous, and no alterations in growth conditions accompany the mutations. Another case, somewhat similar to the last, has recently arisen. It produces alleles giving various lowered expressions of the *Wx* phenotype, and appears to be autonomous although the information on its behavior is too incomplete to allow a full description. The sixth case is one that has been investigated by Sager (1951). It is autonomous, and gives quantitative grades of expression in the endosperm; but the germinal mutations that have been studied all give full or nearly full *Wx* expression, and the mutants are stable. No altered growth conditions appear to accompany these mutations.

Genetic analyses have indicated that all these various mutational changes occur at this one locus in chromosome 9, and yet all show a different kind of mutational behavior. It is evident that each arose in association with a particular type of alteration at the locus, and that different mutation-controlling mechanisms can be involved.

This is especially well illustrated by a comparison of the types of mutations produced by wx^{m-1} and wx^{m-3} and of their controlling mechanisms.

b. The C locus

The contrasts in the kinds of phenotypic expression produced by mutations at c^{m-1} and c^{m-2} have been discussed above. Although several other independent expressions of mutability at this *C* locus have also arisen, the study of them is too incomplete to allow detailed comparisons to be made. With respect to this locus in chromosome 9 it is necessary to mention, however, that in the cultures having mutable loci a heritable factor carried by a chromosome other than 9 has appeared on several occasions. The presence of such a factor results in the production of pigment in the aleurone when the endosperm is homozygous for the well-investigated stable recessive *c*, used for many years in genetic investigations. The pattern of pigment formation differs markedly from that produced by mutations at c^{m-1} or c^{m-2}. It resembles that associated with the factor *Bh* (Blotch), previously studied by R. A. Emerson (1921) and Rhoades (1945b). To complete the discussion of the series at this locus, it may be mentioned that a mutable condition has also arisen involving the expression of *I*, an allele of *C*. Changes in the degree of inhibitory action of *I* occur as a consequence of such mutations.

c. The A_1 locus

A study of changes at the locus of A_1 has contributed some very important information regarding the origin and behavior of mutable loci. For a number of years, a type of control of mutability of the recessive, a_1, has been known. A dominant factor, called Dotted (*Dt*), provokes mutability at the a_1 locus (Rhoades, 1936, 1938, 1941, 1945*a*). In many respects, *Dt* is comparable to *Ac*. It is an activator, for it produces mutations at a_1, just as *Ac* produces mutations at c^{m-1}, c^{m-2}, bz^{m-1}, bz^{m-2}, and wx^{m-1}. Moreover, when *Dt* is absent no mutations occur at a_1; the a_1 locus then gives a stable recessive phenotype. In the presence of *Dt*, mutations occur at a_1 to give mainly the higher alleles of the A_1 phenotypic expression. The time of occurrence of visible mutations at a_1 is usually late in the development of the plant or the endosperm tissues; and they occur in only some of the cells. This results in the presence of dots of the A_1 phenotype. The *Dt* factor has been located by Rhoades in the knob region terminating the short arm of chromosome 9. *Dt* and *Ac* appear not to be the same activator, as plants and endosperm tissues that are homozygous for the recessive a_1 have not shown the dotted-type mutations to A_1 in the presence of *Ac*.

In the early period of this investigation of newly arisen mutable loci, the unexpected appearance of modifications in the knobs, and in the other chromosome elements previously mentioned, of plants that had undergone the breakage-fusion-bridge cycle in their early development, suggested that disturbances in these elements might have been responsible for the initial burst of mutable loci, including the origin of *Ac* itself. It was suspected, therefore, that this cycle might induce alterations in the heterochromatic elements that could initiate a *Dt* factor as they may have originated the *Ac* factor. Once initiated, this factor would activate a_1 to undergo alterations in somatic cells leading to A_1-type expression. The most direct way to induce changes in the heterochromatic elements was considered to be the breakage-fusion-bridge cycle itself. By subjecting tissues to this cycle during their developmental periods, and then examining the matured tissue, this hypothesis could be tested. A preliminary experiment, designed to test for production of mutations of a_1 to A_1 by the breakage-fusion-bridge cycle as an inductor, was performed in 1946. The experiment was repeated in 1950 on a much larger scale. Because this experiment was of particular significance in revealing the mode of origin of mutable conditions, and because it provided evidence about the relation of chromosome organization to genic expression and its control, the details will be given.

The silks of plants homozygous for a_1 and carrying no *Dt* factor received pollen from plants of similar constitution with respect to a_1 and *Dt*. The pollen parents carried one chromosome 9 with a duplicated segment of the short arm. The homologous chromosome 9 was deficient for a terminal segment of the short arm. Newly broken ends of chromosome 9 were produced in some meiotic cells, as diagrammed in Figure 1. Pollen grains of these plants carried either: (1) a deficient chromosome 9, which did not function in pollen-tube growth, (2) a chromosome 9 with a full duplication of the short arm—that is, the homologous chromosome 9, or (3) a chromosome 9 with a newly broken end. Among this last type, duplications or deficiencies of the short arm were present. Those carrying an extensive deficiency were nonfunctional but those carrying a relatively short duplication were better able to compete in functioning than those carrying the full duplication of the short arm. Thus the

majority of the functioning pollen grains of these plants carried a newly broken end of the short arm of chromosome 9 in their nuclei. These chromosomes had undergone the breakage-fusion-bridge cycle since the meiotic anaphase and continued to do so after being incorporated into the primary endosperm nucleus. Either before fertilization or during the development of the kernel, the breakage-fusion-bridge cycle might produce alterations in heterochromatic elements and some of them might include an alteration that would recreate the condition associated with *Dt* action. Mutations at the a_1 locus to give the A_1 phenotype could subsequently appear in the descendant cells. If this should occur early in development of the endosperm, a sector with dots of the A_1 phenotype would be produced. Examination of 95 ears resulting from the preliminary test conducted in 1946 revealed A_1 dots on 15 kernels from 14 different ears. In five of these kernels, more than one A_1 dot was present, and in one restricted region of the kernel in each case. The number of kernels with mutations to A_1 in this trial experiment was lower than anticipated, and the experiment was not expanded the following year. Later, as the probable relation between the origins of mutability and the alterations induced in the knobs or other chromosome elements by the breakage-fusion-bridge cycle became more clearly apparent, the same experiment was conducted in 1950 on a much larger scale. The results were rewarding, for now many kernels were obtained that had one or more A_1 dots. One hundred and twenty such kernels appeared in this second trial, and 24 of them had more than one A_1 spot. One of these kernels had 84 A_1 spots, distributed rather evenly over the kernel. In the other 23, the spots were not distributed at random over the aleurone layer but were restricted to well-defined sectors. In none of these kernels did any large areas of the A_1 phenotype appear. In all cases, the time of mutation, the pattern of mutation, and the type of mutation were much like those produced when the known *Dt* factor is present in endosperms homozygous for a_1. It was obvious that in each case the initial alteration had occurred in the ancestor cell that produced the dotted sector. This initial event was responsible for mutations that occurred at a_1 in some cells during the subsequent development of the endosperm. The observed mutations at a_1, therefore, were not produced directly by the breakage-fusion-bridge cycle but arose secondarily, as a consequence of an event that altered some particular component in the nucleus. It was the alteration of this component that was responsible for the subsequent mutations at the a_1 locus. And this initial alteration was one that imitated the effect produced when the known *Dt* factor is present. It is difficult to avoid the conclusion that a new *Dt*-like factor has been produced in each such case, and that it was created by some event associated with the breakage-fusion-bridge cycle. Unfortunately, the plant grown from the one kernel having 84 dots distributed over the whole aleurone layer did not show any mutations to A_1, nor did mutations appear in the kernels when this plant was crossed to plants homozygous for a_1. The effective alteration probably was present in only one of the two sperms carried in the pollen grain. Because the break in chromosome 9 that initiated the breakage-fusion-bridge cycle was produced at the meiotic anaphase, the event giving rise to the *Dt*-like factor would have had to occur in the subsequent microspore division in order to be incorporated in the two sperm nuclei. An even larger experiment of this same type must be conducted if such a case is to be obtained. It should be mentioned in this connection that the size of the sectors within which A_1 spots appeared graded from large to small, the smaller sectors being most frequent. Also, about three-fifths of the kernels showing mutations to A_1 had only one A_1 spot. These frequencies are to be expected if the creation of a *Dt*-like factor is a consequence of an event, associated with the breakage-fusion-bridge cycle, that has a probability of occurrence in a limited number of mitotic cycles. In order to indicate why the dotted pattern of mutations to A_1 is to be anticipated, rather than any other, it is necessary to review the origin of the previously discovered $Dt - a_1$ mutable condition.

The $Dt - a_1$ mutable condition first appeared on one ear after self-pollination of a plant belonging to the commercial variety known as Black Mexican Sweet Corn. This variety is homozygous for A_1. The recessive a_1 in this case represented a new mutation from A_1, and was associated with the appearance of *Dt*. The original a_1 mutant, known for many years and used in genetic studies, had originally been found to be present in a commercial variety of maize. Both a_1 mutants responded in much the same manner when *Dt* was present in the nucleus. In both cases, the dotted mutation pattern was produced in the presence of *Dt*. The states of the two a_1 mutants thus appeared to be alike. This suggests that the older a_1 mutant may

have been produced by a mechanism similar to that responsible for the origin of the newer a_1 mutant. A *Dt* factor may have arisen at the same time but subsequently been lost from the commercial variety during its propagation, leaving an apparently stable a_1 mutant. The change at *C* that produced c^{m-1} would have behaved quite comparably had *Ac* been absent from the nuclei in the initial gamete carrying c^{m-1}, or had it been removed by crossing before the change at this locus had been detected. If the mutation had been discovered several generations after its origin, and if *Ac* had been removed by a previous cross, it would have appeared to be a newly arisen, stable, recessive *c*. Only after an incidental cross to a plant carrying *Ac* would its potential mutability have been revealed. It is possible, therefore, that many known recessives may prove to be potentially mutable.

The essential similarity of the $Dt - a_1$ system to the $Ac - c^{m-1}$, etc. system is also expressed in the changes in state of a_1 that may occur in the presence of *Dt*. Such changes in state of a_1 have recently been described by Nuffer (1951). They are recognized individually by marked departures in frequency of visible mutations, in types of mutation and in time of occurrence of these mutations. The types of different phenotypic expression produced by mutations at altered states of a_1 are much the same as those produced by a mutable a_1 locus that has appeared in the Cold Spring Harbor cultures. This new mutable a_1 locus, called $a_1{}^{m-1}$, differs from the mutable a_1 studied by Nuffer in that it is autonomous and does not require *Dt* for mutability to be expressed. In this respect, mutability at the A_1 locus behaves like that at the *C* and the *Wx* loci, for both autonomous and activator-controlled mutable conditions may arise.

The origin of $a_1{}^{m-1}$, in a culture carrying mutable luteus, was described previously. It is autonomous, and produces a series of quantitative alleles, many of which are unstable in that they may mutate to give higher or lower levels of quantitative expression. Difference in degree of quantitative expression is only one of the consequences of mutations occurring at $a_1{}^{m-1}$, however. The diversity of phenotypic changes arising from these mutations is so great that an adequate analysis of all the observed types is a large task. They are distinguished not only by quantitative but also by qualitative differences in the anthocyanin pigments formed. Diversity is shown in other respects. For example, some of the mutations giving pale aleurone color are related to changes involving the rate of a particular reaction responsible for pigment formation. Others appear to be related to the absolute amount of pigment that may be produced, regardless of a time factor. This becomes evident when comparisons are made of pigment-forming capacities in plants arising from kernels carrying different mutants of $a_1{}^{m-1}$, each producing a pale color in the aleurone of the kernel. In some cases, such plants are pale in their expression of anthocyanin color throughout their lives. Others are pale in anthocyanin color up to the time of anthesis, when growth of the plant terminates; but in the six or seven following weeks, as the plants mature their ears, the anthocyanin color gradually deepens, becoming intensely dark by the time the ears are mature. The kernels derived from both these two types of plants, however, may be equally pale. The fact that pigment forms late in the development of the kernel, and dehydration of the tissues occurs shortly thereafter, may explain this similarity in color of the kernels.

Other types of mutation occur at $a_1{}^{m-1}$. Some produce sectors of deep color that are rimmed by areas in which the color gradually fades off to colorless, as if a diffusible substance associated with pigment formation had been produced in excess in the mutant sector. The area of diffusion may be very extensive for some mutations, and only slight for others, whereas still other mutations give rise to no such diffusion areas at all. Some of the mutations that result in strong A_1 pigmentation are associated with failure of development or degeneration of some of the aleurone cells within the mutated sector.

Besides the mutational changes at $a_1{}^{m-1}$ that affect the type and amount of pigment formation, a number of other changes occur which affect the subsequent behavior of $a_1{}^{m-1}$. These alterations are termed changes in state, since they affect not only the times when pigment-forming mutations will occur at the locus in future plant and endosperm cells but also the kinds and frequencies of such mutations, and their distributions and their sequences in the development of the tissues.

Any interpretations that attempt to explain the primary action of a specific locus in a chromosome, and how this action may be changed, must take into consideration the facts just enumerated concerning the behavior of this $a_1{}^{m-1}$ locus. It is not reasonable to regard such changes in expression and action as being produced by changes in a single gene—that is, according to the usually accepted concepts of the gene that have

been developed. The evidence suggests, rather, that the observed changes result either from alterations at a locus that has many individual components or from alterations at the locus affecting its relationship to other loci in the chromosome complement. If the latter is true, a combination of loci functions as an organized unit in the production of pigmentation. If such functional organizations exist within the nucleus —and it is reasonable to assume they do—then the large numbers of alleles known to arise at certain loci need not express altered genic action at the identified locus. Rather, any one alteration may affect the action of the organized nuclear unit as a whole. The mode of functioning of various other loci concerned may thereby be modified. In other words, the numerous different phenotypic expressions attributable to changes at one locus need not be related, in each case, to changes in the genic components at the locus, but rather to changes in the mechanism of association and interaction of a number of individual chromosome components with which the factor or factors at the locus are associated. According to this view, it is organized nuclear systems that function as units at any one time in development. In this connection it may be repeated that at a_1^{m-1}, and also at other mutable loci, many of the alterations observed represent changes in the potential for patterns of genic action during development (changes in state). Thus a pattern-controlling mechanism is being altered. If particular nuclear components are formed into organized functional nuclear units, the evidence would suggest that this may happen only at prescribed times in the development of an organism. In this event it may readily be seen that changes in pattern-controlling mechanisms would serve as a primary source of potential variability of genic expression without requiring any changes in the genes themselves.

A few more pertinent facts about the A_1 locus may now be mentioned. Mutability has arisen at this locus independently on a number of different occasions. Several cases have recently appeared in the stocks having *Ds* and *Ac*. Analysis of these cases has not proceeded to a stage where a complete description of their behavior may be given. Both Laughnan (1950) and Rhoades (1950) have found new cases of instability at this locus. Several have appeared in plants derived from kernels that had been aged for some time (Rhoades, 1950). Such aging is known to give rise to chromosomal aberrations as well as mutations; the observed instability may be an expression of one such structural change. Laughnan (1949, 1951) has shown that mutations at the A_1 locus may be associated with the mechanism of crossing over, suggesting again that mutations arise from structural changes at the locus. The crossover studies may elucidate the nature of some of these changes. Not only the cases described in this report but also others have produced evidence converging in support of a hypothesis that mutations originate from structural alterations in chromosome elements. The evidence derived from a study of progressive changes in state of c^{m-1} has shown the close relation between a structural change at a locus and one that so often has been called a "gene mutation." This study will now be described.

Significance of Changes in State of a Mutable Locus: Selected Examples

The foregoing review of the very different types of phenotypic expression that may be produced as a consequence of mutations arising at any one locus, and of the relation between the origin of a mutable condition and the type of mutations expressed, clearly indicates the necessity for caution in attempting to interpret the mutation process as one associated with a "change in a gene." With respect to events occurring at *Ac*, c^{m-1}, c^{m-2}, bz^{m-1}, bz^{m-2}, and wx^{m-1}, it has been established that a mechanism capable of producing chromosomal breaks at the locus is associated with the mutation-producing process. It could be argued from the evidence so far presented that these cases fall into a special category, and that what they may indicate regarding the mechanism of mutation at these mutable loci may not be used to interpret the mutation process in general. Knowledge gained from a study of the changes in state of c^{m-1} has shown, however, that no line may be drawn between those events at a locus that produce detectable chromosomal alterations and those that give rise to mutations but produce no readily detectable chromosomal alterations.

The origin of c^{m-1} by a transposition of *Ds* to the normal *C* locus has been discussed above. The state of *Ds*, when first transposed to the *C* locus, was one that produced many detectable chromosome breaks at this locus and few mutations to *C*. When plants having this state of c^{m-1} were crossed to plants that were homozygous for the stable recessive *c*, the majority of the resulting kernels showed this relationship. Some of these kernels, however, had sectors with higher rates of mutation to *C* and lower rates of detectable

chromosome breaks. In some sectors, no chromosome breaks were evident; but often a high frequency of mutation to *C* had occurred. There were also a few kernels on these ears that had this pattern throughout the kernel. When found, such kernels were selected from the ears (as well as others that showed changed mutational patterns). The plants grown from them were again crossed to plants homozygous for the stable recessive *c*. The kernels on the resulting ears now showed the types and frequencies of the different detectable events at c^{m-1} that had been observed in the kernel from which the plant had arisen. It was possible to determine in this manner that a heritable change had occurred at c^{m-1}. It was this change which was responsible for the altered frequencies of expression of the detectable events that subsequently occurred at this locus. That the altered response, in each isolated case, arose from a change at c^{m-1} and was not produced by a change at *Ac*, was determined by testing the responses of these c^{m-1} isolates with different isolates of *Ac*, each having a known type of action. *Ac* controlled, in each test, the time and place of the event occurring at c^{m-1}, but not its type. Because, in each case, the change in behavior of c^{m-1} was heritable, it must have arisen by an event that produced an alteration at this locus. It is this heritable altered condition that has been termed an altered state of the locus.

The various altered states of c^{m-1}, as previously mentioned, arise only when *Ac* is also present in the nuclei, and only at the times in development when mutations or chromosome breaks may occur at c^{m-1}. For our purposes, the most instructive of the changed states are those giving reciprocal frequencies of chromosome breaks and mutations to *C*. A series of isolates, each showing a particular relation between these two events at c^{m-1}, has been studied. The isolates ranged from those showing no mutations to *C* or only a very occasional one, but having a very high frequency of detectable chromosome breaks, to those showing a high frequency of mutations to *C* and no detectable breakage events or only an occasional one. The states of c^{m-1} that give high frequencies of chromosome breaks are unstable, for other altered states may be produced as a consequence of events at the locus, but only, as emphasized above, when *Ac* is also present in the nucleus. A particular state of c^{m-1} remains constant if maintained in plants having no *Ac*. The state of c^{m-1} giving no detectable chromosome breaks, and a correspondingly high frequency of mutations to *C*, is very stable with respect to the absence of breakage events. Had the state of c^{m-1} been of this type when it first arose, there would have been no opportunity to discover that the chromosome-break-producing mechanism and the mutation-producing mechanism were related. If chromosome breaks are not exhibited by a mutable locus, therefore, it cannot be argued that because of this the basic mechanism producing the mutations must be different from that known to operate at c^{m-1} and at the other *Ac*-controlled mutable loci. The evidence obtained from this study of the origin and subsequent behavior of altered states of c^{m-1} argues, rather, for similarities if not identities in the basic mechanism.

Another type of change in state of c^{m-1} should be mentioned, although it occurs infrequently. It is detected by a much altered expression of the mutation at this locus that affects aleurone color. The frequency of origin of such states of c^{m-1} is so very low as to suggest that they represent entirely new modifications at this locus, comparable to the original inception of a mutable condition at any locus. They may well represent just such new inceptions, for these are to be expected in view of the fact that very different types of mutational behavior are exhibited at this same locus by c^{m-1} and c^{m-2}, both of which are *Ac*-controlled.

All the *Ac*-controlled mutable loci exhibit changes in state. These are characterized by changed relative frequencies of the different recognizable types of mutations that occur. In other words, as we have already seen, the types of mutation produced in *Ac*-controlled mutable loci are related to the state of the mutable locus itself. The time and place during development of occurrence of mutations, on the other hand, are controlled by the state and dose of *Ac*; and therefore alterations in them are related to changes in state of *Ac* rather than to changes in state of the mutable locus. The changes in state of *Ac* have been described. The autonomous mutable loci also undergo changes in state, as described previously for the a_1^{m-1} locus. In this group, however, the controller of the time and place of occurrence of mutations is a component of the locus itself. Consequently, the changes of state that arise at these loci are reflected in changes in the control of time and place of mutations as well as in the type or types of mutation that may occur, and also in the time and place of occurrence of each such mutation if several types are produced. Thus there is a much more diverse

group of altered states associated with changes at any particular locus in the autonomous group. The general similarities between the autonomous and the *Ac*-controlled mutable loci are nevertheless striking.

Extent of Instability of Genic Expression in Maize

The discussion so far has mentioned a number of different mutable conditions at known loci in maize. In order to show that the phenomenon is much more prevalent than the particular cases described would indicate, some additional observations of changes in genic expression may be discussed briefly. Not only have *C*, *I*, *Bz*, *Wx*, A_1, and A_2 become mutable during the course of these studies, but instability of other known dominants has been noted. These are *R*, *Pr*, *Yg*, *Pyd*, *Y*, and possibly *B* and *Pl*, although the evidence for the last two is observational and not genetic. Some previously unknown dominant loci have also become unstable. These are associated with various chlorophyll-determining factors, endosperm-starch-controlling factors, aleurone-color factors, growth-controlling factors, etc. Instability has arisen at the loci of some of the known recessives such as *wx*, *yg*, and the special case a_1 described previously. Instability at the loci of the recessives *y* and *p* also appears to have arisen on several independent occasions. Genetic tests were not made, however, to determine the association of the instability with the known loci. Instability arising at recessive loci is recognized by mutations to or towards the expression of the dominant allele. It may be concluded, therefore, that many of the known recessive alleles are potentially capable of expressing action that is characteristic of the dominant alleles.

The expression of instability of various factors in maize is probably far more common than has been suspected in the past. Until recently, only a few such cases had been reported in the literature. One of the earliest recognized was that occurring at the *p* locus (pericarp and cob color, chromosome 1), studied by R. A. Emerson and his students (for literature citations, see Demerec, 1935) and recently being studied by Brink and his students (1951, and personal communication) and by Tavcar (personal communication). Reported cases of instability at the a_1 and the *wx* loci have been mentioned previously in this discussion. In addition, Rhoades (1947) has studied instability at the *bt* locus (brittle endosperm, chromosome 5) and has reported two cases involving chlorophyll characters that appeared in the progeny of irradiated seed (Rhoades and Dempsey, 1950). Fogel (1950) has been investigating instability at the *R* locus (aleurone, plant, and pericarp color, chromosome 10); and Mangelsdorf (1948) has reported instability at the *Tu* locus (Tunicate, chromosome 4). It is believed that critical examination will uncover many such cases in maize, and that they will involve many different loci.

Mutable Loci and the Concept of the Gene

It will be noted that use of the term gene has been avoided in the foregoing discussion of instability. This does not imply a denial of the existence within chromosomes of units or elements having specific functions. The evidence for such units seems clear. The gene concept stems from studies of mutation. That heritable changes affecting a particular reaction, or the development of a particular character, in an organism arise repeatedly and are associated with a change of some kind occurring at one specific locus or within one specific region of a chromosome, has been established. This knowledge has been responsible for the development of a concept requiring unitary determiners. It cannot be denied, in the face of such evidence, that certain loci or regions in the chromosomes are associated in some manner with certain cellular reactions or with the development of particular phenotypic characters. This is not the major questionable aspect of current gene concepts. The principal questions relate to the mode of operation of the components at these loci, and the nature of the alterations that affect their constitution and their action. Within the organized nucleus, the modes of operation of units in the chromosomes, of whatever dimensions these may be, and the types of change that may result in specific alterations in their mode of action, are so little understood that no truly adequate concept of the gene can be developed until more has been discovered about the function of the various nuclear components. The author agrees with Goldschmidt that it is not possible to arrive at any clear understanding of the nature of a gene, or the nature of a change in a gene, from mutational evidence alone. At present, the most we know about any "gene mutation" is that a heritable change of some nature has occurred at a particular locus in a chromosome, and that any one locus is somehow concerned with a certain chemical reaction, or with a certain restricted phenotypic expres-

sion, or even with the control of a complex pattern of differentiation in the development of a tissue or organ. The various types of known mutation, each showing unitary inheritance, obviously reflect various levels of control of reactions and reaction paths. It is necessary to consider these various widely different levels of unitary control and how they may operate in the working nucleus, and also to consider the nature of the changes that can affect their operation. It is with the nature of such heritable changes, the conditions that induce them, and their consequences, that this report has been concerned. Various levels of unitary control, as witnessed by inheritance behavior, are evident from the study. That genes are present in the chromosomes and that they function to produce a specific type of reactive substance will be assumed in this discussion, even though such a restricted assumption may prove to be untenable. The knowledge gained from the study of mutable loci focuses attention on the components in the nucleus that function to control the action of the genes in the course of development. It is hoped that the evidence may serve to clarify some aspects of gene action and its control. Some of the interpretations of the author, based on this evidence, have been stated or implied at various points in the previous discussion, and may now be summarized.

The primary thesis states that instability arises from alterations that do not directly alter the genes themselves, but affect the functioning of the genic components at or near the locus of alteration. The particular class to which a mutable locus belongs is related to the particular kind of chromatin substance that is present at or near the genic component in the chromosome. It is this material and the changes that occur to it that control the types and the rates of action of the genic components. Thus the basic mechanism responsible for a change at a mutable locus is considered to be one that is associated with a structural alteration of the chromatin materials at the locus. The mechanism that brings about these changes is related to the mitotic cycle; and it may involve alterations of both sister chromatids at the given locus. Some of these alterations may immediately result in the expression of an altered phenotype, a "gene mutation." Others produce modifications controlling the type of events that will occur at the locus in future cell and plant generations. Still others produce changes of a more extensive type, such as duplications and deficiencies of segments of chromatin in the vicinity of the locus. With regard to these conclusions, the evidence presented in the discussion of changes in state of $c^{m\text{-}1}$ may be recalled.

Behavior and Action of *Ac* in Cell and Plant Generations

The interpretations given above deal with the organization and the kinds of events that occur at genetically detectable loci in the chromosomes. The next level of consideration deals with mode of operation of the nucleus in controlling the course of events during development. Do these studies suggest a mode of operation, or at least one component in the operative system? It is believed that the behavior of *Ac* may be of importance in such a consideration. With reference to $c^{m\text{-}1}$, for example, it has been shown that mutations to *C* occur at particular times and places in development, under the control of the particular state and dose of *Ac*. It has also been shown that *Ac* is unstable, for changes arise affecting its dosage action, and changes also occur in its location in the chromosome complement. It has not been explained, however, that the time of occurrence of these changes at *Ac* during development is also a function of the particular state and dose of *Ac* that is present in a tissue. With any particular *Ac*, and any particular dose of this *Ac*, or with any combination of different isolates of *Ac*, the time when these events will occur to *Ac* is a function of the single or combined action of the *Ac* factors. An example of this may now be given.

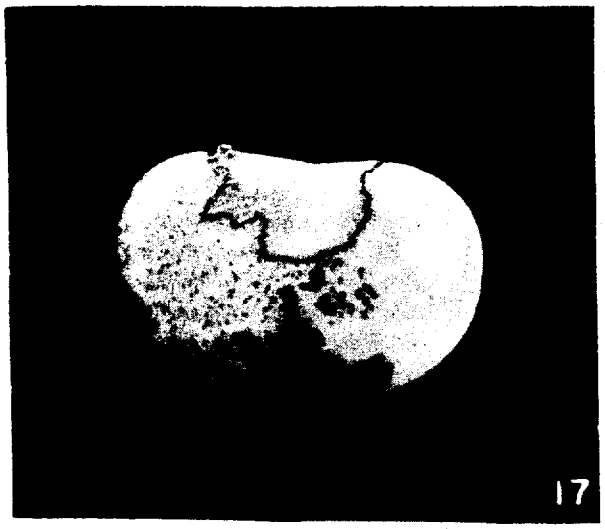
One plant having an *Ac* factor at the same location in each homologue of a pair of chromosomes, and carrying *l*, *Sh*, *Bz*, *Wx*, and *Ds* (standard location) in both chromosomes 9, was crossed to a number of plants homozygous for *C*, *sh*, *bz*, and *wx* and carrying no *Ds* or *Ac*. On all the many ears resulting from these crosses, approximately 90 per cent of the kernels were sectorial with reference to the time of occurrence of *Ds* events. Pollen from this same plant was placed on silks of plants having other combinations of markers in chromosome 9, and similar types of sectorial kernels appeared. The sectoring was produced by segregations, occurring in the earliest nuclear divisions of the endosperm, that involved the controller of the time of occurrence of *Ds* events, that is, the Activator. The action of *Ac* in these different sectors resembled that occurring either (1) when no *Ac* is present, (2) when a sharply decreased dose of *Ac* is present, or (3) when a sharply increased dose of *Ac* is present. Illustrations that will make this relation-

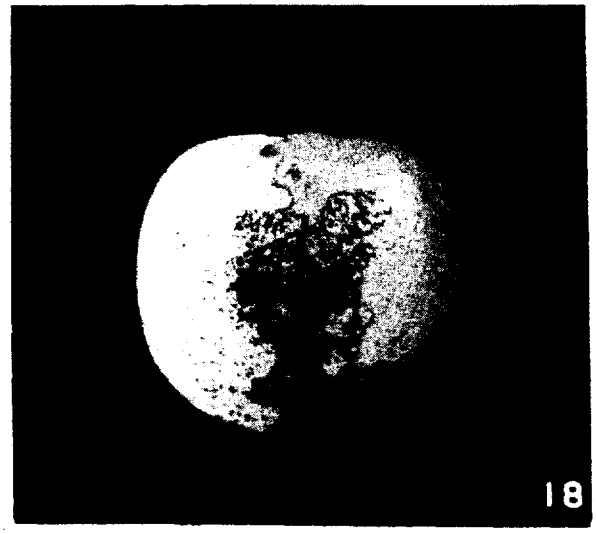
ship clear are shown in the photographs of Figures 17 to 22. In a definite fraction of the cases, a chromosome break at *Ds* was associated with segregations of *Ac* action. The mechanism responsible for this precise, somatically occurring segregation for *Ac* action was very likely the same as that responsible for the origins of germinal changes in action of *Ac*, as well as changes in its position in the chromosome complement. Such changes have been mentioned earlier in this report. It can be seen that if this same type of segregation occurred within some cells early enough in the development of a plant to be incorporated in a microspore or megaspore nucleus, the altered state or location of *Ac*, or both, would be recoverable in the plant that subsequently resulted from the functioning of the male or female gametophyte arising from such a spore. Such an early-timed event would allow for isolation and subsequent study of the transpositions and changes in state of *Ac*.

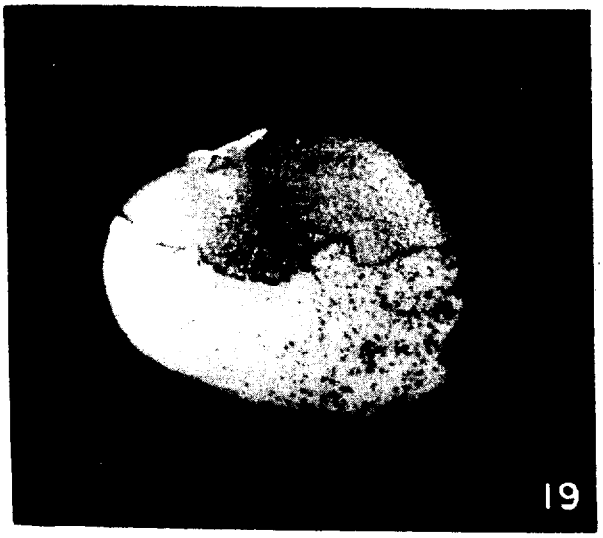
A study was initiated to determine the nature of the changes that occur at *Ac* by an analysis of the *Ac* constitutions in the gametes of plants having an *Ac* factor at the same location in each member of one pair of chromosomes, that is, in plants homozygous for *Ac* in allelic positions. In these plants the *Ac* factors were alike in state, and the homozygous condition was produced by self-pollination of plants having one *Ac* factor. Both chromosomes 9 in these plants carried the stable factors *c*, *sh*, and *Wx*. No *Ds* was present in these chromosomes. To test for *Ac* inheritance, these plants were crossed by plants having no *Ac* but carrying *C*, *Sh*, *wx*, and *Ds* in both chromosomes 9 (standard locations of *Ds* and identical states). If no changes had occurred to *Ac*, all the kernels on the resulting ears should be variegated. Similar variegation patterns, produced by sectors showing the *c*, *sh*, and *Wx* phenotype, should be present, because *Ac* would initiate chromosome breaks at *Ds* in the *C*, *Sh*, *wx*, *Ds*-carrying chromosome 9 contributed by the male parent. With the exception of a few kernels, just such conditions were realized on these ears. A photograph of one such ear appears in Figure 23. It will be noted that the majority of kernels on this ear show very similar patterns of variegation. A few kernels that differ from the majority are completely colored, with no colorless sectors of any size. In them, no *Ds* breaks at all occurred. A few other atypical kernels show an altered timing of the breakage events at *Ds*. In them such events occurred either much earlier or very much later in development of the endosperm.

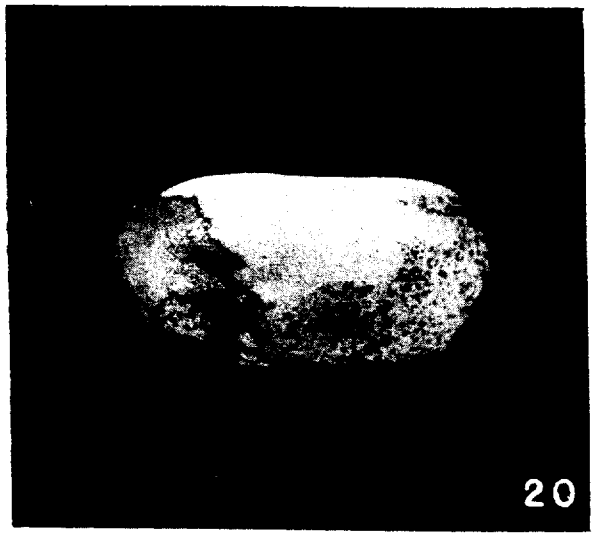
A study was made of the *Ac* constitution in plants derived from selections of all the different types of kernels appearing on such ears. In the plants coming from the kernels showing altered variegation patterns, it was necessary to determine the subsequent behavior of *Ac*; and in the plants coming from the nonvariegated kernels it was necessary to determine the presence or absence of *Ac*, and the presence or absence of *Ds* in the *C*, *Sh*, *wx*-carrying chromosome. The results of this study may be summarized. In the plants derived from the majority class of kernels, a single *Ac* factor was present. Its state was similar to that present in the parent plant (more than 25 cases studied). The *Ac* constitution in the plants derived from the nonvariegated kernels was most instructive. In 19 plants, no *Ac* was present. In 17 plants, two nonlinked *Ac* factors were present. In six plants, an *Ac* factor inherited as a single unit was present, but it gave a dose action equivalent to two doses of the *Ac* factor in the parent plant. In the plants derived from kernels that showed very late-occurring *Ds* events, either two nonlinked *Ac* factors were present (5 cases), or a single *Ac* factor was present giving a dose action greater than that of the *Ac* factor in the parent plant (3 cases).

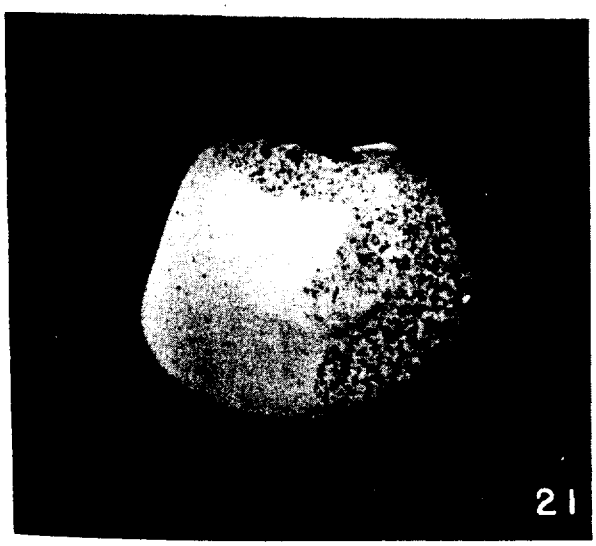
FIGS. 17 to 22. Photographs of kernels illustrating the somatic segregations of *Ac* that may occur very early in the development of a kernel. These kernels arose from the cross of plants (♀) carrying *C*, *bz* and no *Ds* in each chromosome 9 and having no *Ac* factor, by plants carrying *I*, *Bz*, and *Ds* (standard location) in chromosome 9 and also carrying *Ac*. For phenotypes expected from breaks at *Ds*, see descriptions accompanying Figures 10 to 15. In Figures 17 and 18 there are 4 large sectors in each kernel: one is *C bz* (above in Figure 17, to right in Figure 18), one is *I*, non-variegated (to right in Figure 17, upper left in Figure 18), one is characterized by late occurring *Ds* breaks, producing speckles of the *C bz* genotype (left in Figure 17, lower left in Figure 18), and one shows that numerous *Ds* breaks occurred earlier in the development of the kernel (lower segment in Figure 17, middle segment in Figure 18). The kernel in Figure 19 has 3 sectors: one that is *C bz*, one with few specks of the *C bz* genotype and one with many specks of the *C bz* genotypes. Figure 20 shows a kernel with 3 sectors: one that is *C bz*, one that is wholly *I Bz* and one having many specks of the *C bz* genotype. Figure 21 shows a kernel with two sectors: one with many specks of the *C bz* genotype and one with few such specks. Figure 22 shows a kernel with five sectors: a large *C bz* sector (lower left), a large sector having many specks of *C bz* (upper), a large sector showing many larger *C bz* areas (upper right), a small sector with few *C bz* specks (middle), and a sector of *I Bz* with no *C bz* specks (lower right).

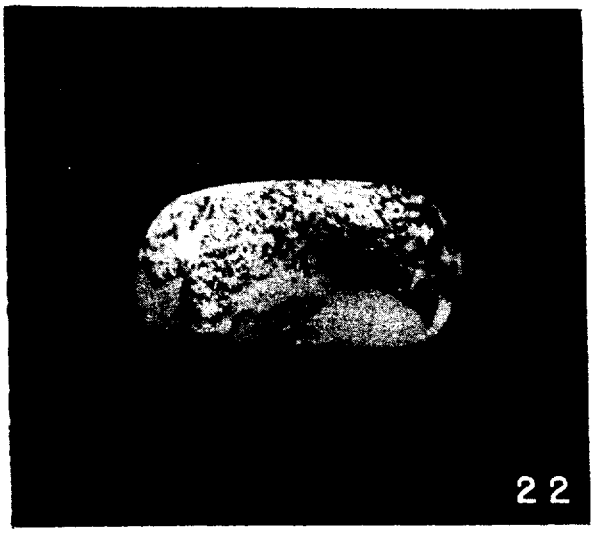

17


18


19


20


21


22

From this analysis it is clear that all the aberrant kernels on ears of the type shown in Figure 23 were produced because of some alteration of *Ac* that had occurred in cells of the parent plant. The reason that no *Ds* breaks were detected in some of these kernels is related either to the absence of *Ac* in the endosperm or to the presence of a marked increase in the dose of *Ac*. It will be recalled that the female parent contributed two gametophytic nuclei to the primary endosperm nucleus. If each nucleus carried two *Ac* factors, or a single *Ac* factor with a double-dose action, the endosperm would have either four *Ac* factors, or two *Ac* factors equivalent in action to four *Ac* factors. In such kernels, the high dose of *Ac* so delayed the time of occurrence of *Ds* breaks that none took place before the endosperm growth had been completed.

In order to verify the analyses of *Ac* constitution in some of these cases, tests were continued for another generation. For example, if a plant contains two nonlinked *Ac* factors, the gametic ratios approach 1 two-*Ac* : 2 one-*Ac* : 1 no-*Ac*—that is, a three-to-one ratio for the presence of *Ac*. On ears derived from crosses in which such plants are used as male parents, the kernels with one or with two doses of *Ac* may be distinguished because of clearly seen differences in the time of response of *Ds* to *Ac* doses. Therefore, some of the kernels considered to have two *Ac* factors and others considered to have only one *Ac* factor were selected from the test ears. The plants grown from them were again tested for gametic ratio of *Ac*. In each case, verification was realized. The gametic ratios produced by the latter plants approached 1 one-*Ac* : 1 no-*Ac*, whereas those produced by the former approximated 3 with one or two *Ac* factors to 1 with no *Ac*.

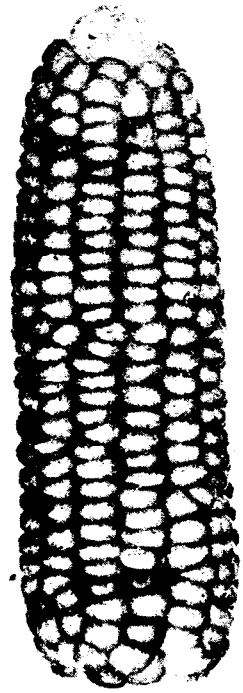


FIG. 23. Photograph of an ear derived from a plant having two identical *Ac* factors located at allelic positions in an homologous pair of chromosomes. This plant carried *c* in each chromosome 9. The ♂ parent, having no *Ac*, introduced a chromosome 9 with *C* and *Ds* (standard location). The majority of the kernels are similarly variegated for sectors of the *c* genotype due to breaks at *Ds* that occurred in the *C Ds* chromosome during the development of the kernels. Note the few fully colored kernels in which *c* sectors are absent, and also the several kernels that show large sectors of the *c* genotype.

The above-described series of tests, and still others that have been concerned with the time and type of changes occurring at *Ac*, have made it possible to understand the nature of its inheritance patterns. It has been found that, with any particular state or dose of *Ac*, the time of occurrence of changes of *Ac* is controlled by *Ac* itself. If, with a particular *Ac* state, the time of such changes is delayed until late in the development of the endosperm, then all the kernels should show this same late timing. This is known to occur with many of the isolates of *Ac*. As the photograph in Figure 23 has shown, however, a few aberrant kernels may be present on some of these ears. Some internal or external alteration in environmental conditions may have caused these few early-occurring changes at *Ac*. No attempts have been made, however, to study conditions that might alter the time of such changes.

If these tests for determining the inheritance behavior of *Ac* had not been made, considerable confusion might have arisen. This would certainly have been true had states of *Ac* giving relatively early changes been used in the initial inheritance studies. It must be stated that just such a situation has been observed. States of *Ac* giving aberrant gametic ratios have arisen. It is now realized that this is to be anticipated. It has been determined that the reason for the difference in patterns of inheritance between an *Ac* isolate that gives clear-cut mendelian gametic ratios and one of its modified derivatives, that gives aberrant gametic ratios, is related to the time in the development of the sporogenous or gametophytic cells at which such changes in *Ac* arise. With reference to the gametic constitutions that

will be produced, the time when these changes occur is most critical. If they occur in somatic divisions before the meiotic mitoses, or in the male or female gametophytes, an apparently unorthodox inheritance pattern for *Ac* will result. If they occur late, that is, in the endosperm tissues—which act in this connection like a continuation of the development of the gametophyte—then no such confused pattern of inheritance will arise. The gametic constitutions will then closely approximate those predicted for mendelizing units. In the study of *Ac* inheritance, it was necessary to make selections for these latter states of *Ac*. A few exceptions with regard to the time of changes at *Ac* may occur in some cells, even with such selected states of *Ac*. It was the analysis of *Ac* constitutions in plants derived, in cell lineage, from those cells in which such exceptional timing of changes at *Ac* had occurred, that provided the information leading to appreciation of the somatic origins of altered states and locations of *Ac*.

Confused patterns of inheritance behavior of mutable loci have been described in the literature many times. The ratios obtained have often been so irregular that no satisfactory formulation of the nature of the inheritance patterns could be derived. This would be just as true of some of the autonomous mutable loci in maize if attention had not been given to altered states and their behavior. Two examples may illustrate this. Both a_1^{m-1} and a_2^{m-1}, when first discovered, produced many mutations and changes in state very early in the development of the plant. The plant, therefore, was sectorial for the altered conditions at these mutable loci. The sectors were present in the tassel. When pollen was collected without reference to the sectors present, and placed on the silks of plants carrying the stable a_1 or a_2 alleles, the kinds of kernels appearing on the resulting ears, and their frequencies, were not readily analyzable in terms of mendelian ratios. No such difficulty arises, however, when similar tests are made for gametic ratios in plants derived from those kernels on the original test ears that show only very late-occurring mutations. The inheritance pattern is now of the obvious mendelian type, for mutations and changes in state are mainly delayed until after meiosis and gamete formation. As with *Ac*, the selection of states of autonomous mutable loci that produce very late-occurring mutations makes it possible to examine the inheritance behavior of such loci, freed from the apparent confusion resulting from early-occurring modifications at the locus, which can distort the expected mendelian ratios.

Is the Behavior of *Ac* a Reflection of a Mechanism of Differentiation?

We now return to the original question. What is the significance of the somatically occurring changes at *Ac*, and the changes in state that occur at the autonomous mutable loci? Do they suggest the presence of nuclear factors that serve to control when and where certain decisive events will occur in the nucleus? With regard to *Ac*, it is known that the events leading to its loss, to increase or decrease of its dosage action, or to other changes involving its action or position in the chromosome complement, are related; and that they appear as the consequence of a mitotic event, controlled in time of occurrence by the state and dose of *Ac* itself. Sister nuclei are formed that differ with respect to *Ac* constitution, as the photographs of Figures 17 to 22 illustrate. Because of this somatically occurring event involving *Ac*, the *Ac*-controlled mutable loci will differ markedly as to the time when mutational events will occur at them, or as to whether or not any such events will occur at all in the cells arising from the sister cells. This precise timing of somatic segregations effects a form of differentiation, for it brings about changes in the control of occurrence and time of occurrence of genic action at other loci, and does so differentially in the progeny of two sister cells. This likewise applies to the autonomous mutable loci; but in these cases the controller of the time and place of appearance of genic activity is a component of the locus itself.

The process of differentiation is basically one involving patterns of action arising in sequential steps during development and affecting the types of activities of definitive cells. The ultimate expression of component parts of an organism represents the consequence of segregation mechanisms involving the various cellular components. The part played by any one component of the cell in this segregation system can not be divorced from that played by any other component. It is possible, however, to attempt to examine the various components in order to determine their respective relationships and the sequential events that involve them. Embryological studies have contributed much to our knowledge of the segregation of cytoplasmic components. The segregation of nuclear components is less well understood, although some outstanding examples are known. These examples show segregations or losses of obvious components of the nucleus—that is, of whole chromosomes or easily seen parts of chromosomes. The segregation or loss of smaller components, not readily visible on microscopic

examination, may well be one of the mechanisms responsible for the nuclear aspects of control of the differentiation process. The phenomenon of variegation, as described here and observed in many other organisms, may be a reflection of such a segregation mechanism—exposed to view be- the timing of events leading to a specific type of genic action is "out-of-phase" in the developmental path. Variegation may represent merely an example of the usual process of differentiation that takes place at an abnormal time in development. Viewed in this way, it is possible to formulate an interpretation of the part played by the nuclear components in controlling the course of differentiation.

This interpretation considers that the nucleus is organized into definite units of action, and that the potentials for types of genic action in any one kind of cell differ from the potentials in another kind of cell. In other words, the functional capacities of the nuclei in different tissues or in different cells of a tissue are not alike. The differences are expressions of nonequivalence of nuclear components. This nonequivalence arises from events that occur during mitotic cycles. The differential mitotic segregations are of several types. Some involve controlling components, such as *Ac*, and produce sister nuclei that are no longer alike with respect to these components. As a result, the progeny of two such sister cells are not alike with respect to the types of genic action that will occur. Differential mitoses also produce the alterations that allow particular genes to be reactive. Other genes, although present, may remain inactive. This inactivity or suppression is considered to occur because the genes are "covered" by other nongenic chromatin materials. Genic activity may be possible only when a physical change in this covering material allows the reactive components of the gene to be "exposed" and thus capable of functioning.

A mechanism of differentiation that requires differences in nuclear composition in the various cells of an organism finds considerable support in the literature. The most conspicuous example is in *Sciara*, where a thorough cytological and genetical analysis has been made. (For reviews, see Metz, 1938, and Berry, 1941.) It is known, in this organism, at just what stage of development differences in nuclear composition will arise; and, with regard to the X chromosome, it is known what element in the chromosome controls the differential behavior. This element is at or near the centromere of the chromosome (Crouse, 1943). Furthermore, differential segregations of the B-type or accessory chromosome have been found to occur in a number of plants. (For reviews of literature to 1949, see Müntzing, 1949.) Numerous other examples are known of differential segregation involving whole chromosome complements, certain types of chromosomes of a complement, or, occasionally, a certain component of a chromosome. (For literature citations see Melander, 1950; White, 1945, 1950; Berry, 1941.) Whether the differential segregations involve whole complements of chromosomes, individual chromosomes, individual parts of chromosomes that can be seen, or submicroscopic parts of chromosomes, may well be a matter of degree rather than type. Certainly, the evidence for differential segregation is not wholly negative.

With regard to mechanisms associated with differentiation and genic action, an additional factor may be mentioned. The part played by the doses of component elements in the chromosomes appears to be of considerable importance. First, a number of genetic factors associated with known loci produce measurable quantitative effects that are related to dose: the higher the dose the greater the effect. Such dosage actions, probably reflecting rates of reaction, are familiar to all geneticists, and some of them have been reviewed in this study of mutable loci. Dosage controls of the *Ac* type, affecting the time of action of certain other factors carried by the chromosomes, has been less well appreciated. A third type of dosage action has made itself evident in these studies. In some aspects, however, it resembles the action of different doses of *Ac*. In the study of the autonomous a_2^{m-1} mutable locus, a number of mutants appeared, particularly on self-pollinated ears, showing a pale aleurone color. Study of the behavior of these pale mutants has revealed the following. Some of them produce pale-colored aleurone in one, two, and three doses and give no evidence of instability in the expression of the phenotype. (One and two doses are obtained by combinations of the pale-mutant allele with the stable a_2 allele.) That this stable expression may be deceptive is shown by the dosage effects of other similarly appearing pale-producing isolates derived from mutations at a_2^{m-1}. These may give pale aleurone color, and no indication of instability, in three and two doses; but with one dose something unexpected occurs. The kernels show a colorless aleurone in which mutations to deep aleurone color appear. Still other isolates give pale color in three doses, but in one or two doses produce the colorless background with deep-colored mutant areas. In these cases, it

is clear that some of the mutations at $a_2^{m\text{-}1}$ giving pale color and appearing to be stable are stable only because of some dosage action produced by a mutation of the original $a_2^{m\text{-}1}$ to the pale-producing type.

The study of dose-provoked actions in the pale mutants mentioned above and those of *Ac* have given some indication of the importance of dosage action in affecting genic expression. The original isolate of $a_2^{m\text{-}1}$ did not give evidence of such striking dosage action. When present in one, two, or three doses, it gave rise to colorless kernels in which mutations, mainly to a deep color and occasionally to a pale color, appeared. The graded series of dosage action exhibited by the various pale mutants derived from $a_2^{m\text{-}1}$ is very much the same as the graded series exhibited by the various isolates of *Ac*. In these cases, it appears as if each isolate is composed of a specific number of reactive subunits and that the dosage expressions are related to the total number of such units that are present in the nucleus. Although these graded dosage effects may be visualized on a numerical basis, it is not claimed that such an interpretation is necessarily the correct one. The large differences in dosage expression exhibited by the various isolates of *Ac*, and also the various isolates of the pale mutants derived from $a_2^{m\text{-}1}$, nevertheless appear to follow such a scheme.

Why different doses of components of the chromosomes function as they do in controlling developmental processes takes us to another level of analysis that is not under consideration here. A relation to rates of particular reactions can be suspected. It is tempting to consider that changed environmental conditions may well alter otherwise-established rates of reaction, and thus initiate alterations in the nuclear components at predictable times, leading to strikingly modified phenotypic expression. Just such effects have been observed by students of developmental genetics. They have shown that alterations of environmental conditions at particular times in development can lead to predictable changes in the subsequent paths of differentiation.

Consideration of the Chromosome Elements Responsible for Initiating Instability

It will be recalled that this study of the origin and behavior of mutable loci was undertaken because a large number of newly arisen mutable loci appeared in the progeny of plants in which an unusual sequence of chromosomal events had occurred—that is, the breakage-fusion-bridge cycle. Striking similarities in the patterns of behavior of these mutable loci were immediately noticed. It was the pattern of behavior, rather than the change in expression of the particular phenotypic character, that was obviously of importance. This pattern, revealed in all cases, stemmed from an event occurring at mitosis, which altered the time and frequency of mutations that would subsequently occur in the cells derived from those in which this event occurred. It was noticed that sister nuclei could differ in these respects—and sometimes reciprocally, as if the mitotic event had resulted in an increase in one nucleus of a component controlling the mutation time or frequency, and a decrease of this component in the sister nucleus. It was also noted that the change in phenotypic expression—that is, the mutation—likewise resulted from a mitotic event; and that the mutation itself and changes of the controller of the mutation process could result from the same mitotic event: one cell showing the mutation, the sister cell showing an altered condition with respect to control of future mutations in the cells derived from it.

Further, it may be recalled that the mechanism which resulted in the appearance of newly arisen mutable loci—that is, the breakage-fusion-bridge cycle involving chromosome 9—gave rise to numerous obvious alterations of the heterochromatic materials, in other chromosomes of the complement as well as in chromosome 9. It was also demonstrated that the effect of a known activator, *Dt*, located in the heterochromatin of the chromosome-9 short arm, and producing a very definite pattern of mutations of the otherwise stable a_1 locus in chromosome 3, could be recreated independently and on a number of different occasions in cells of a tissue in which the breakage-fusion-bridge cycle was in action. The combined observations and experiments point to elements in the heterochromatin as being the ones concerned with differential control of the times at which certain genes may become reactive. It is believed that somatic segregations of components of these elements may initiate the process of nuclear control of differentiation.

On the basis of these interpretations and those given in the previous section, it becomes apparent why a large number of newly arisen mutable loci appeared in the self-pollinated progeny of plants that had undergone the chromosome type of breakage-fusion-bridge cycle. This cycle induced alterations in the heterochromatin. These alterations changed the organization of the heterochro-

matic chromosome constituents and probably also, in many cases, the doses of their component elements. Changes were induced in these heterochromatic elements at times other than those at which they would normally occur during differentiation. This resulted in changes in the times in development when their action on specific chromatin material, associated with genic components of the chromosome, was expressed. The altered timing of their actions was consequently "out-of-phase" with respect to the timing that occurs during normal differentiation. This was made evident by the appearance of a "mutable locus." The "mutable locus" is thus a consequence of the alteration of an element of the heterochromatin produced by the breakage-fusion-bridge cycle. Once such an "out-of-phase" condition arises, others may subsequently appear because of the physical changes in the chromatin that occur at the mutable locus, leading, at times, to transpositions of this chromatin to new locations, as described earlier. In their new locations, these transposed chromatin elements continue their specific control of types of genic action but now affect the action of the genic components at the new locations.

Relation of "Mutable Loci" to "Position-effect" Expressions in Drosophila and Oenothera

In a previous publication (McClintock, 1950) the author has suggested that the position-effect variegations in *Drosophila melanogaster* and the variegations observed in many other organisms, including those associated with the mutable loci here described, are essentially the same. An adequate discussion of the interrelations would require more space than can appropriately be given here. Attention will be drawn, therefore, only to a few relevant facts, which may serve to indicate why this conclusion has been reached. In the first place, a number of different types of position-effect expression are found in *Drosophila* (for review and literature citations, see Lewis, 1950). In maize, comparable types of instability expression have appeared. In *Drosophila*, some of the variegations appear to result from loss of segments of chromosomes. This applies to those cases where the expression of the dominant markers, carried by the chromosome showing the "position-effect" phenomenon, is absent in some sectors of the organism. The extent of the deficiency varies, but it includes in each case the region adjacent to the heterochromatic segment with which many of the variegation types of position-effect expression are known to be associated. It may be recalled that such deficiencies are produced in maize when *Ds* is present.

That heterochromatic elements of the chromosomes of *Drosophila* undergo breakage events in somatic cells is suggested by the study of "somatic crossing over" in this organism (Stern, 1936). The appearance of the abnormally timed exchanges between chromosomes is conditioned by the presence of certain Minute factors, for example, *M(1)n*, much as the occurrence of structural aberrations at certain loci in maize (i.e., *Ds*, wherever it may be) is dependent on the presence of *Ac*.

Of particular significance for comparative purposes is the study of Griffen and Stone (1941) on the induction of changes in the position-effect expression in *Drosophila* of the white-eye variegation, w^{m5}. The w^{m5} case arose through an X-ray-induced translocation of the segment of the left end of the X chromosome at 3C2 (the w^+ locus) to the heterochromatic region of chromosome 4. Males carrying w^{m5} were X-rayed, and the progeny examined for changes in the variegation expression of the eye mottling. Many such changes were found. Studies of these cases were continued in order to determine the nature of the events associated with the changes. In all cases, the new modification in the phenotypic expression of the w^+ locus was found to be associated with a translocation, which placed the segment of the left end of the X chromosome, from 3C2 to the end of the arm, at a new location. In many cases, the new position was to a euchromatic region of another chromosome, and yet variegation persisted. Some of the new positions, however, gave rise to apparent "reversions" to a wild-type expression. Individuals having these "reversions" were X-rayed, and variegation types again appeared in the progeny. Here also the variegation was shown to be associated with a translocation involving the left end of the X chromosome at 3C2, from the location in the "reversion" stock to a new location—again, sometimes a euchromatic region. It may be suspected that the maintenance of variegation potentialities in all these cases was associated with the presence of a segment of heterochromatin of chromosome 4 that remained adjacent to the w^+ locus when the successive translocations occurred. This would not readily be detected in the salivary chromosomes. The presence of such "inserted" heterochromatin could be responsible for the continued expression of variegation at the w^+ locus in repeated translocations. If such was

the case, then the resemblance to the maize cases, described in this report, is obvious. The appearance of "reversions," and the subsequent appearance of variegation after X-radiation of individuals carrying such "reversions," might seem to present a contradiction. On the basis of an analysis of the cases described by Griffen and Stone, the writer believes that no contradiction is involved. This analysis has suggested that the timing of variegation-producing events during development is, in part, a function of the relative distance of the translocated segment—i.e., the left end of the X chromosome—from the centromere of the chromosome that carries it: the farther removed the segment is from the centromere, the later in the development the variegation-producing events will occur. In the "reversions," this segment has been placed close to the end of one arm of a chromosome. The reappearance of variegation occurs when the segment is translocated to a position closer to a centromere. Another factor is also associated with the timing of the variegation-producing events. This is the Y chromosome. When the Y is absent, the areas of altered phenotype are larger than when it is present, indicating an earlier timing of the variegation-producing events. It may be noted in this connection that some of the cases of "reversions" are only apparent reversions. In XY constitutions they appear to give a stable wild-type expression but in XO constitutions, the eyes show a light speckling of the altered phenotype. With the latter constitution variegation occurs, but only very late in the development of the eye. The similarity of this effect of the Y chromosome to that of dosage action of *Ac* is apparent in these cases as well as in many others in *Drosophila* that have been examined.

In a recent report, Hinton and Goodsmith (1950) gave an analysis of induced changes at the bw^D (Dominant brown eye) locus in chromosome 2 of *Drosophila*. This case was considered to be a stable-type position effect. It arose originally through the insertion of an extra band next to the salivary-chromosome band where bw^+ is located. Males carrying bw^D were irradiated and crossed to wild-type females. The offspring (9,757 individuals) were examined for changes in the bw^D expression. Twenty-one individuals showing the wild-type expression appeared in the F_1, and progeny was obtained from one-third of them. A study of the inheritance behavior of each modification was undertaken, and a study was also made of the salivary-gland chromosomes. From these studies it was clear that the modifications arose from changes that occurred in the vicinity of the bw^D locus and involved the inserted band. In four cases, restoration of the wild-type expression followed removal of this band. In two cases, it followed separation of this band from the bw^+ band by translocations. In one case, no obvious change in the salivary chromosomes was noted, but nevertheless a change in phenotypic expression had occurred. Of considerable importance, also, was the appearance, in some of these cases, of somatic instability of expression of the bw^+ phenotype. Variegation began to appear. It had never been observed in the brown-Dominant stock itself. It may be noted that the changes in the bw^D expression are associated with types of chromosomal alterations which are much the same as those proposed to account for changes in phenotypic expression at some mutable loci in maize.

A further resemblance between *Drosophila* and maize will be mentioned. In *Drosophila*, many of the translocations, inversions, and duplications are believed to be associated with the formation of dominant-lethal effects. In maize, a number of dominant lethals have arisen from transpositions of *Ds*. Some produce defective growth of the endosperm and embryo; others affect the development of the embryo but not the endosperm; and still others affect the capacity of the embryo to germinate, without affecting its morphological characters. Over half the newly arisen transpositions of *Ds* that are of this latter type have not produced viable plants, owing to lack of germination of the embryos in the kernels.

There are similarities between the maize cases and a case in *Drosophila pseudoobscura* described by Mampell (1943, 1945, 1946). In this *Drosophila* case, a heterochromatic element appeared to be associated with the initiation of instability at another locus, which in turn led to changes in chromosome organization and to numerous changes in genic action at various loci in the chromosome complement. These changes were expressed both somatically and germinally.

The position-effect behavior reported in *Oenothera* (Catcheside, 1939, 1947a, b) is much like that of *Ds*. The chromosomal events responsible for the observed types of change in phenotypic expression may be the same in the two organisms. In *Oenothera* as in maize, gross changes in chromosome constitution arise, such as duplications and deficiencies of segments of the chromosome involved. Similar cases in other organisms undoubtedly exist. It is probable, however, that

the lack of a critical mode of detection of a chromosome breakage mechanism has been responsible for the apparent delay in reporting such cases in connection with studies of somatic variegation and mutable loci. Also, because changes in state occur that involve reduction in the frequency of chromosome breaks, and because such breaks lead to lethal gametes, it is probable that states of a mutable locus producing some detectable breaks are rapidly eliminated from a population, leaving a state of the mutable locus that produces few or no such events to be propagated.

It has been argued that the variegation types of position effect in *Drosophila* usually do not give rise to germinal mutations, and that they belong, therefore, to a separate category of instability expression. Since some variegation position effects do give rise to germinal changes, this argument could in any case be only partially applicable. However, whether or not germinal changes arise is not considered relevant in the interpretation developed here. The time and place of occurrence of such changes is related to controls, existing in the nucleus. The differentiation mechanism described above should effect controls that would exclude the germ lines from undergoing many changes, but should allow numerous alterations in the soma that would lead to altered patterns of genic expression. Whether or not a particular somatically expressed pattern of genic action—for example, the distribution of pigment—arises from mutations at a "mutable locus" or from the action of a particular "stable" allele of the locus cannot be decided by using the criterion of presence or absence of germinal mutations. The important consideration is when, where, and how the patterns of genic action are controlled and eventually expressed.

The combined evidence from many sources suggests that one should look first to the conspicuous heterochromatic elements in the chromosomes in search of the controlling systems associated with initiation of differential genic action in the various cells of an organism; and secondarily to other such elements, which are believed to be present along the chromosomes and to be either initially or subsequently involved in the events leading to differential genic action. Evidence, derived from *Drosophila* experimentation, of the influences of various known modifiers on expression of phenotypic characters has led Goldschmidt (1949, 1951) to conclusions that are essentially similar to those given here.

The conclusions and speculations on nuclear, chromosomal, and genic organization and behavior included in this report are an outgrowth of studies of the instability phenomenon in maize. They are presented here for whatever value they may have in giving focus to thoughts regarding the basic genetic problems concerned with nuclear organization and genic functioning. Until these problems find some adequate solution, our understanding and our experimental approach to many phenomena will remain obscured.

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